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# IN THE UNITED STATES COURT OF FEDERAL CLAIMS
# OFFICE OF SPECIAL MASTERS

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|---|---|---|
| IN RE: CLAIMS FOR VACCINE INJURIES RESULTING IN AUTISM SPECTRUM DISORDER, OR A SIMILAR NEURODEVELOPMENTAL DISORDER, | * | Autism Master File |
| Various Petitioner(s) | * | |
| v. | * | **REPLY BY AMICUS SMITHKLINE BEECHAM CORPORATION D/B/A GLAXOSMITHKLINE TO PETITIONERS' RESPONSE TO MERCK AND AMICUS CURIAE RE: NON-PARTY DISCOVERY** |
| SECRETARY OF HEALTH AND HUMAN SERVICES, | * | |
| Respondent. | * | |
| * * * * * * * * * * * * * * * * * * * * * * * * * | * | |

SmithKline Beecham Corporation d/b/a GlaxoSmithKline ("SB"), in its capacity as amicus curiae, files this reply to Petitioners' Response to Merck and Amicus Curiae re: Third-Party Discovery ("Petitioners' Response") to show the following:

## INTRODUCTION

Petitioners ask the Special Master to take an unprecedented step in issuing a subpoena seeking expansive discovery from a non-party vaccine manufacturer, yet they can point to nothing more than their own expedient interpretation of the Vaccine Court's rules to justify the request. They argue that Congress intended the Vaccine Act to alleviate the liability exposure of vaccine companies, but that it never envisioned that these companies would be wholly exempt from discovery in a Vaccine Court proceeding. With all due respect, the statutory framework and legislative history that will be discussed below makes clear that Congress was concerned with precisely these types of expensive litigation burdens, not just final judgments. In fact, there is no precedent for guiding the Special Master in addressing many of the substantive and logistical problems raised by Petitioners' request because the Vaccine Act was designed to exclude the vaccine companies from proceedings in this Court.

Keeping this important legislative backdrop in mind, Merck and the amici have each demonstrated in their prior briefs that the statutory and judicial standards that stand as a precondition to any discovery in this Court raise a high bar when applied to parties to a Vaccine Court proceeding. That burden is necessarily and additionally elevated when the object of discovery is a non-party—especially a non-party like Merck and the amici whom the Vaccine Act was enacted to protect. The only reasonable interpretation of Congress' intent is that resort may be made to discovery—at least as to third-parties covered by the Vaccine Act—only when there is a "gap" in the proof necessary to establish entitlement to compensation and the particular information that is lacking cannot be acquired another way.

Petitioners deftly try to convert the factual "gap" explained by Merck and the amici into a wholly unsubstantiated "scientific gap" theory. According to Petitioners, they are entitled to have access to the records of vaccine manufacturers whenever the available science is insufficient to support their burden of showing causation. That interpretation flies in the face of the Vaccine Act's structure and purpose. As SB explained in its amicus brief at 3-4, the Act envisions that Petitioners will assemble the available proof in a compensation proceeding, and the Special Master will determine whether it proves causation.

Petitioners maintain that the available studies themselves have identified "scientific gaps" that Petitioners assert can only be filled in with documents that may or may not exist in the vaccine companies' files. Specifically, they point to the October 2001 Report of the Institute of Medicine ("IOM Report") and remarks found on the website for FDA's Center for Biologics Evaluation and Research ("CBER"). Yet they fail to address the numerous recent studies that ***are*** publicly available—and with good reason. The wealth of available scientific data has closed the gaps in science identified in the 2001 IOM Report, but Petitioners understandably find it lacking since the legitimate science tends to refute, rather than substantiate, their theory of causation. Petitioners' dissatisfaction with the scientific data that is publicly available provides no basis for subjecting the vaccine companies to the significant burden of responding to broad discovery requests in a proceeding that Congress deliberately exempted them from being part.

## ANALYSIS AND AUTHORITIES

### A. Plaintiffs Have Failed to Articulate a "Necessity" that Would Warrant Broad and Invasive Access to the Vaccine Companies' Files

Petitioners' argument as to why the documents they seek are "reasonable" and "necessary" starts with the premise that "the Special Master and petitioners [do not] have all the relevant evidence needed to conclude the causation analysis." Petitioners' Response at 3. From there, they proceed to dismiss the documents provided by the HHS Secretary thus far as providing only "a paucity of causation evidence." *Id.* Thus, Petitioners urge, "the Special Master needs to look elsewhere for the information." *Id.* Since "one may sensibly assume that Merck . . . is a reasonable place for the Special Master to turn," Petitioners conclude they have established the showings of necessity and reasonableness required to entitle them to discovery. *Id.*

The foundational assumption of Petitioners' analysis—that they are presumptively entitled to discover "all relevant evidence needed to conclude the causation analysis"—is where they first go astray of the Vaccine Act. The standard they articulate speaks to traditional discovery—not the presumptively limited role of discovery placed upon Vaccine Court proceedings. *Compare see* FED. R. CIV. P. 26(b)(1) ("Parties may obtain discovery regarding any matter, not privileged, that is relevant to the claim or defense of any party . . . ."), *with* 42 U.S.C. § 300aa-12(d)(2)(E) (mandating that the Vaccine Court rules "provide for limitations on discovery and allow the special masters to replace the usual rules of discovery in civil actions in the United States Court of Federal Claims"). The fact that Merck may have "relevant" information does not make it reasonable or necessary to require Merck to seek out and produce wide-ranging materials from its extensive files.

Although Petitioners suggest that they "need only describe the information gaps identified in the respondent's own document production to provide a sufficient showing of necessity for the authorization to issue . . . the requested subpoena," Petitioners' Response at 3, notably, they point only to the IOM Report dated more than two years ago and remarks taken from the CBER website to identify those "information gaps." *Id.* at 4. Petitioners suggest that those "gaps" might possibly be filled in by information in the control of the vaccine companies. In fact, in the last two years, there have been numerous articles and studies on the very subjects that Petitioners itemize from the 2001 IOM Report. In the Research and Articles Summary and Appendix that is separately bound and being submitted contemporaneously, SB has summarized and attached abstracts and articles reporting on a number of recent studies and scientific data gathered in connection with research into the existence of any causative link between thimerosal and autism-related disorders. There have also been statements issued by various national and international agencies charged with vaccine safety after research has been presented to those agencies on those same subjects. As the lengthy appendix attests, there is a wide body of scientific knowledge on the subject, and Petitioners should be required to identify the specific gaps in that information—not resort to a two-year old IOM Report—before they can begin to surmount the compelling presumptions against third-party discovery from vaccine companies.

### B. Merck's "Familiarity" With its Vaccine Product Goes to "Relevance" Not "Necessity"

Petitioners argue that "Merck's familiarity with the Recombivax product that it designed, tested, manufactured, and distributed for over a decade is an additional reason for allowing the requested discovery." Petitioners' Response at 5. If Petitioners' proposed standard were adopted, it would be hard to imagine a case involving a vaccine-related injury where that same showing could not be made. Again, Petitioners resort to relevance discovery standards, which are inapplicable in this Court.

Nonetheless, Petitioners attempt to draw support from a prior decision of this Court, *Wittner v. Sec'y Dept. of Health and Human Servs.*, 43 Fed. Cl. 199 (1999), in which a Special Master permitted the testimony of a consulting expert witness over the objection of the petitioner in that case. The consulting expert had been the treating physician for the injured child. The fact that non-party evidence was allowed in that case provides no basis for the discovery sought here. There is no claim that any vaccine company has particularized knowledge of the specific injuries or treatment of any petitioner in this case—only that the vaccine companies "might" have some materials in their possession that Petitioners hope might compromise the profusion of available scientific data that points away from any causal connection. Notably, Petitioners themselves do not claim to be aware that this information actually exists; they speculate only that if they could review the vast files of the vaccine companies, they might find something useful. But without even a hint as to what that information might be, their request to plow through hundreds of thousands of documents to see if it even exists is decidedly unreasonable.

### C. The Balancing of the Respective Interests and Burdens Clearly Favors the Vaccine Companies

Petitioners appear to believe that it is "necessary" to go directly to the files of the vaccine companies since the production of PLAs by the government has taken so long. They argue that the delay in production means delay in compensating so many injured children. Petitioners' Response at 8-9. But the solution they offer—shifting the burden of large-scale discovery to the vaccine companies—would not provide any real remedy and certainly is not grounds for putting the vaccine companies to this monumental task under the circumstances. As explained in the prior briefing, Petitioners are no more likely to get these documents more quickly from the vaccine companies than from the government, which has already been far down the road in compiling the PLA documents and negotiating trade secret redactions with the vaccine companies. Although Merck suggested potential ways to speed up the process—such as allowing the government to produce the PLA documents as currently redacted so that Petitioners can determine if the trade secrets are really worth fighting about—Petitioners do not even respond. If they truly want the majority of PLA documents sooner, it is curious that they will not consider more productive and less intrusive means to obtain them.

Finally, in their balancing-of-the-equities analysis, Petitioners suggest that the Vaccine Act was designed to prevent vaccine companies from liability exposure, not litigation burdens. Petitioners Response at 1, 9-10. In this assumption, they are simply wrong. Congress was clear that the litigation costs were an equally compelling reason for the Compensation Program: "Lawsuits and settlement negotiations can take months and even years to complete. ***Transaction costs—including attorneys' fees and court payments—are high***." H.R. Rep. 99-908, at 6-7, *reprinted in* 1986 U.S. CODE, CONG. & ADMINISTRATIVE NEWS ("U.S.C.C.A.N.") 6344, 6347 (emphasis added). This sought-after discovery will force the vaccine companies to incur precisely the types of litigation transactional costs that Congress intended be avoided.

## CONCLUSION

Petitioners have failed to show that the discovery they request is reasonable or necessary under the circumstances. Their request for a subpoena should be denied.

Respectfully submitted,

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*Application for admission to the United States Court of Federal Claims pending

## CERTIFICATE OF SERVICE

I hereby certify that on December 29, 2003, I served the foregoing Reply by Amicus SmithKline Beecham Corporation d/b/a GlaxoSmithKline to Petitioners' Response to Merck and Amicus Curiae re: Non-Party Discovery on the following individuals via facsimile and email transmission:

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December 29, 2003

**VIA HAND DELIVERY**

Clerk of the Court
U.S. COURT OF FEDERAL CLAIMS
717 Madison Place, NW
Washington, DC 20005-1011

Re: *In Re: Claims for Vaccine Injuries Resulting in Autism Spectrum Disorder, or in a Similar Neurodevelopmental Disorder v. Secretary of Health and Human Services*; In the United States Court of Federal Claims, Office of the Special Master, Autism Master File

Dear Clerk:

Enclosed please find the original and three copies each of the following:

- Reply By Amicus SmithKline Beecham Corporation d/b/a GlaxoSmithKline to Petitioners' Response to Merck and Amicus Curiae re: Non-Party Discovery; and

- Research and Articles Summary and Appendix.

Please file the enclosed in your usual manner, returning file-stamped copies of each to me via the courier provided.

Thank you for your attention to this matter. If you have any questions, please do not hesitate to contact me at (512) 536-5216.

Very truly yours,

Marcy Hogan Greer

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MHG/lak
Enclosures

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**IN THE UNITED STATES COURT OF FEDERAL CLAIMS**
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| SECRETARY OF HEALTH AND HUMAN SERVICES, | * * * | |
| Respondent. | * | |
| * * * * * * * * * * * * * * * * * * * * * * * * * | * | |

**RESEARCH AND ARTICLES SUMMARY AND APPENDIX**

Respectfully submitted,

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## INDEX AND BIBLIOGRAPHY

A. Research and Articles Summary


1. Burbacher, TM, et al. Mercury levels in blood and brain of infant monkeys exposed to thimerosal [Abstract]

2. Clarkson TW, et al. Human exposure to mercury: the three modern dilemmas. *J. Trace Elements Exper. Med.* 2003;16:321-43.

3. Clarkson TW, et al. The toxicology of mercury --- current exposure and clinical manifestations. *N. Eng. J. Med.* 2003;349:1731-37.

4. Geier MR and Geier DA. Thimerosal in childhood vaccines, neurodevelopmental disorders, and heart disease in the United States. *J. Am. Phys. and Surgs.* 2003; 8(1):6-11.

5. Holmes AS, et al. Reduced levels of mercury in first baby haircuts of autistic children. *Int'l J. of Toxic.* 2003;22:277-85.

6. Hviid A, et al. Association between thimerosal-containing vaccine and autism. *JAMA* 2003;290(13):1763-66.

7. Madsen KM, et al. Thimerosal and the occurrence of autism: Negative ecological evidence from Danish population-based data. *Pediatrics* 2003;112(3);604-06.

8. Magos L. Neurotoxic character of thimerosal and the allometric extrapolation of adult clearance half-time to infants. *J. Applied Toxicology* 2003;23:263-69.

9. Nelson KB and Bauman ML. Thimerosal and autism? *Pediatics* 2003; 111(3):674-79.

10. Pichichero ME, et al. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: a descriptive study. *Lancet* 2002;360:1737-42.

11. Statement from the Committee on Safety of Medicines, Further Data Support Safety of Thiomersal in Vaccines. UK Medicines Control Agency, February 12, 2003.

12. Stehr-Green P, et al. Autism and thimerosal-containing vaccines: Lack of consistent evidence for an association. *Am. J. Prev. Med.* 2003;25(2):101-06.

13. Verstraeten T, et al. Safety of thimerosal-containing vaccines: A two-phased study of computerized health maintenance organization databases. *Pediatrics* 2003;112(5):1039-48.

14. WHO Global Advisory Committee on Vaccine Safety. Statement on thiomersal. August 2003.

**CERTIFICATE OF SERVICE**

I hereby certify that on December 29, 2003, I served the foregoing Research and Articles Summary and Appendix on the following individuals via facsimile and email transmission:

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A

## RESEARCH AND ARTICLES SUMMARY

As mentioned in SB's reply brief, Petitioners' eight alleged "gaps," listed in bullet point fashion on page 4 of their Response, are considered, *seriatim* and in the context of available scientific information, below. The referenced abstracts or articles are included in the attached bibliography for the Special Master's convenience:

**1. "'The data regarding toxicity of low doses of thimerosal and ethyl-mercury are very limited, and only delayed-type hypersensitivity reactions have been demonstrated.'"[1]**

Since the IOM Report, the following studies have appeared in peer-reviewed publications:

- Pichichero ME, et al. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: a descriptive study. *Lancet* 2002;360:1737-42.

Dr. Pichichero and colleagues measured mercury levels in blood, urine and stool samples from 40 infants who received thimerosal-containing vaccines and compared them to 21 control infants receiving thimerosal-free vaccines. In addition to demonstrating that infants rapidly excrete a substantial portion of thimerosal-derived mercury in their feces, the researchers found that the "amounts of mercury in the blood of infants receiving vaccines formulated with thimerosal are well below concentrations potentially associated with toxic effects."

- Magos L. Neurotoxic character of thimerosal and the allometric extrapolation of adult clearance half-time to infants. *J. Applied Toxicology* 2003;23:263-69.

This article noted a significant difference between ethylmercury and methylmercury in terms of their ability to cross the blood-brain barrier. The author noted that it appeared from large dose poisoning data that "ethylmercury is less toxic than methylmercury" based on the amount necessary to produce the toxic effect.

- Clarkson TW, et al. The toxicology of mercury --- current exposure and clinical manifestations. *N. Eng. J. Med.* 2003;349:1731-37.

In this article, the authors compared the clinical toxicologic features of mercury vapor, methylmercury, and ethylmercury found in fish, dental amalgams, and vaccines, respectively. Concerning the vaccine issue, the authors noted the differences between the effects of methylmercury and ethylmercury and concluded:

> [I]n the two-month periods between vaccinations (at birth and at two, four and six months), all of the mercury should have been excreted, so that there is no accumulation.

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[1] Petitioners' Response at 4 (quoting *Immunization Safety Review: Thimerosal Containing Vaccines and Neurodevelopmental Disorders*, Institute of Medicine, October 2001 ["IOM Report"] at 3, 27).

This finding of no accumulation of mercury in blood with successive administration of thimerosal-containing vaccines has been demonstrated in the Burbacher, et al. study of non-human primate infants, discussed below.

- Clarkson TW, et al. Human exposure to mercury: the three modern dilemmas. *J. Trace Elements Exper. Med.* 2003;16:321-43.

Here, the same authors discussed the same issues in considerably more detail. As respects thimerosal in vaccines, they conclude: "Ethyl mercury and therefore thimerosal would appear to be less toxic in humans than methyl mercury compounds."

**2. "There is a need for 'far more evidence of the risks and benefits associated with thimerosal-bearing vaccines.'"[2]**

This quote is ostensibly lifted from the IOM's discussion of the "Public Health Response" (IOM Report, p. 7), which was the appropriate place for the IOM to focus on balancing risks and benefits. However, Petitioners have misquoted the Institute. The precise and complete statement of the IOM is: "There is a need for[3] more evidence on the risks and benefits associated with thimerosal-containing vaccines, biological, and pharmaceutical products in use in the United States and elsewhere." This "gap" has also been filled since October 2001.

- WHO Global Advisory Committee on Vaccine Safety. Statement on thiomersal. August 2003.

The IOM's expressed concern about risks/benefits "elsewhere" (which Petitioners omitted) is demonstrated in this position paper of the GACVS, which was established in 1999 by the World Health Organization to respond "promptly, efficiently and with scientific rigour to vaccine safety issues of potential global importance." After considering data presented by reknowned thimerosal researchers, including Dr. Pichichero, as well as the article by Geier and Geier discussed below, the GACVS determined that "there is no reason on the grounds of safety to change current immunization practices with thiomersal-containing vaccines, since ***the benefit outweighs any unproven risks***." (emphasis supplied.)

- Statement from the Committee on Safety of Medicines, Further Data Support Safety of Thiomersal in Vaccines. UK Medicines Control Agency, February 12, 2003.

This Committee considered two UK epidemiological studies and the Pichichero study (discussed above). The CSM Chairman stated: "The balance of benefits and risks of thiomersal-containing vaccines therefore remains ***overwhelmingly*** positive." (emphasis supplied.)

[2] Petitioners' Response at 4 (purportedly quoting IOM Report at 7).

[3] Note the absence of the adjective "far" in the IOM Report.

3. **"The IOM 'is unaware of risk assessments of thimerosal in pharmaceutical products' and recommends risk-based research."[4]**

The risk/benefit studies referred to in 2 above are also applicable here.

4. **"The report discusses at length the lack of data regarding the toxicity or safety of ethyl mercury, the primary constituent of thimerosal."[5]**

By reiterating the same "gap" with a different characterization, Petitioners appear to be itemizing a greater number of gaps than ever existed. This "gap" is essentially the same as the one discussed in #1. The studies referenced above (Pichichero, et al., Magos, and the two Clarkson, et al. papers) speak directly to this point.

5. **"The report further details the lack of information about low doses of thimerosal, particularly noting the absence of toxicity data for the doses of thimerosal found in the pediatric vaccine schedule."[6]**

The 2002 Pichichero study, discussed above at #1, provides precisely the kind of data the IOM said was lacking. It is a study of exposure of infants who received the low dose of thimerosal that is in fact found in the vaccines.

- Burbacher, TM, et al. Mercury levels in blood and brain of infant monkeys exposed to thimerosal [Abstract]

This recent study in non-human infant primates compared the distribution of mercury in newborn monkeys following intramuscular administration of thimerosal-containing vaccines as compared to oral methylmercury ingestion. Dr. Burbacher and colleagues concluded that "EPA guidelines for methylmercury exposure may not provide an accurate assessment of the public health risk to children receiving thimerosal-containing vaccines."

6. **"The IOM explicitly recognizes the gaps in science by recommending a number of biomedical,[7] clinical, epidemiological, and basic science research areas in order to develop the evidence."[8]**

Summarized in Box ES-1 (IOM Report, pp. 14-15) are the IOM's recommendations of "a diverse public health and biomedical research profile" consisting of Epidemiological Research, Clinical Research and Basic Science Research. What has transpired since the IOM Report is outlined below.

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[4] Petitioners' Response at 4 (quoting IOM Report at 9).

[5] *Id.*

[6] *Id.*

[7] Petitioners have confused the classifications intended by the IOM by suggesting that "biomedical" research is a separate category.

[8] Petitioners' Response at 4.

## Epidemiology

Epidemiological studies have been completed with respect to thimerosal:

- Stehr-Green P, et al. Autism and thimerosal-containing vaccines: Lack of consistent evidence for an association. *Am. J. Prev. Med.* 2003;25(2):101-06.

Dr. Stehr-Green and colleagues performed an ecological study analyzing the reported increases in autism in California, Sweden and Denmark over comparable periods of time. Sweden and Denmark were chosen because in those countries, childhood vaccines have been thimerosal-free since 1993. Both Sweden and Denmark reported increases in the number of diagnosed cases that continued, and even accelerated, after the 1993 removal of thimerosal from childhood vaccines. Recognizing that there are limitations on ecological analyses, the authors concluded that the evidence to date and their data "are not consistent with the hypothesis that increased exposure to Thimerosal-containing vaccines are responsible for the apparent increases in the rates of autism in young children being observed worldwide."

- Madsen KM, et al. Thimerosal and the occurrence of autism: Negative ecological evidence from Danish population-based data. *Pediatrics* 2003;112(3);604-06.

These investigators performed the same type of analysis as did the Stehr-Green study but focused solely on Denmark. They note that "the thimerosal-containing vaccine was gradually phased out meaning that the incidence rates should decline gradually if thimerosal has any impact on the development of autism. However, an increase (rather than a decrease) in the incidence rates of autism was observed."

- Hviid A, et al. Association between thimerosal-containing vaccine and autism. *JAMA* 2003;290(13):1763-66.

This study also focused on Denmark but took a different approach. It is a cohort study comparing the numbers of children diagnosed with autism who were vaccinated with thimerosal-containing vaccines to those who were vaccinated without thimerosal. They found that the risk for autism did not differ significantly between the two groups. They also found no evidence of a dose-response relationship (where those who got higher doses of thimerosal were at increased risk for autism). The authors concluded that "our results are not compatible with the hypothesis of a causal association between thimerosal and autistic-spectrum disorders."

- Verstraeten T, et al. Safety of thimerosal-containing vaccines: A two-phased study of computerized health maintenance organization databases. *Pediatrics* 2003;112(5):1039-48.

This study was a retrospective cohort study of neurodevelopmental disorders in three HMOs in which assessments were made at different times during development based on the amount of thimerosal received. The study concluded: "No consistent significant associations between [thimerosal-containing vaccines] and neurodevelopmental outcomes were found" among the HMOs.

- Geier MR and Geier DA. Thimerosal in childhood vaccines, neurodevelopmental disorders, and heart disease in the United States. *J. Am. Phys. and Surgs.* 2003; 8(1):6-11.

The lead author of this article is well known to this Court. *See Daly v. Secretary of HHS,* No. 90-590V, 1991 WL 154573, n.11 (Chief Spec. Mstr. Golkiewicz): "[T]he court admonishes Dr. Geier to reconsider his role, from a moral standpoint, as a witness under this Program."

The authors claim that their analyses provide "strong epidemiological evidence for a link between mercury exposure from thimerosal-containing vaccines and neurodevelopmental disorders." However, these analyses have been severely criticized by the American Academy of Pediatrics ("Study fails to show a connection between thimerosal and autism"), the National Immunization Program ("The researchers inadequately described the methods they used, making it impossible to determine exactly what was done and how the results should be interpreted. . . . There are a number of weaknesses in this analysis, including an apparent misunderstanding among the authors regarding VAERS reporting requirements.") and GACVS ("[T]he article does not provide a sufficient scientific basis for changing the WHO policy in respect of thiomersal-containing vaccines.").

Among these concerns was the Geiers' use of the Vaccine Adverse Event Reporting System ("VAERS").[9] As the CDC explained in a recent report:

> Passive surveillance systems (e.g., VAERS) are subject to multiple limitations, including underreporting, reporting of temporal associations or unconfirmed diagnoses, and lack of denominator data and unbiased comparison groups. Because of these limitations, determining causal associations between vaccines and adverse events from VAERS reports is usually not possible.

CDC. Surveillance for safety after immunization: Vaccine Adverse Event Reporting System [VAERS] --- United States, 1991-20001. *MMWR* 2003;52[SS-1]:1-24. The IOM has also expressed concern that "VAERS and other case reports submitted to the committee are useful for hypothesis generation, but they are generally inadequate to establish causality." IOM Report, p. 59.[10]

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[9] VAERS is a passive surveillance system meaning that reports are voluntarily submitted by those who witness the adverse events, including practitioners, parents, hospitals, even attorneys.

[10] Other Geier articles suffer from the same deficiencies. *See, e.g.*, Geier MR and Geier DA. Neurodevelopmental disorders after thimerosal-containing vaccines: A brief communication. *Exp. Biol. Med.* 2003;228(6): 660-64; Geier DA and Geier MR. An assessment of the impact of thimerosal on childhood neurodevelopmental disorders. *Pediatr. Rehabil.* 2003;6(2):97-102.

### Clinical Research

For clinical research, the IOM recommended three types of studies: (a) how children metabolize and excrete heavy metals, particularly mercury; (b) modeling of ethylmercury exposures, including the incremental burden of thimerosal with background mercury from other exposures; and (c) "careful, rigorous and scientific investigations of chelation when used in children with neurodevelopmental disorders, especially autism." IOM Report, p. 15. Several studies fit these categories:

- Pichichero, et al. *See* #1 above.

This previously described study specifically investigated the levels of mercury in the blood of infants administered thimerosal-containing vaccines and its subsequent excretion in feces.

- Holmes AS, et al. Reduced levels of mercury in first baby haircuts of autistic children. *Int'l J. of Toxic.* 2003;22:277-85.

These investigators compared the baby hair of 94 children eventually diagnosed with Autism Spectrum Disorder (ASD) to the baby hair of 45 controls. Lab testing determined the mercury content in the hair of the ASD children to be low compared to that of controls. The authors speculate that if mercury is not in the hair, it is still in the body and they "***presume*** that a portion of the tissue mercury retention is sequestered in the central nervous system." (emphasis added).

### Basic Science Research

Here, the IOM focused on two specific categories: (a) research to identify an alternative to thimerosal "for countries that decide they need to switch"[11] and (b) research in animal models on neurodevelopmental effects of ethylmercury. IOM Report, p. 12. As respects animal models, studies are underway which have not yet resulted in published results. Abstracts of those studies have been presented at two International Meetings for Autism Research (IMFAR) in November 2001 and November 2002. Another animal model study by Burbacher, et al. (discussed above) has been abstracted and presented recently to the Advisory Committee on Immunization Practices. Copies of the abstracts of animal studies pertaining to thimerosal are included in this appendix.

**7. "'Additional studies to fill in gaps in our knowledge, such as whether the regressive subtype of autism is causally related to thimerosal in vaccines, is warranted.'"[12]**

The website for the Center for Biologics Evaluation and Research (CBER) states that the "U.S. Public Health Service agencies have collaborated with various investigators to initiate further studies to better understand any possible health effects from exposure to thimerosal in

[11] Petitioners cannot be interested in research concerning preservatives other than thimerosal since they need to prove a causal connection to thimerosal-containing vaccines.

[12] Petitioners' Response at 4 (quoting www.fda.cber.gov, "Frequently Asked Questions").

vaccines." www.fed.gov/cber. Since the government is the Respondent here, it should be the source of the information sought by the Petitioners.

- Nelson KB and Bauman ML. Thimerosal and autism? *Pediatics* 2003; 111(3):674-79.

This commentary relates to the claim that autism is a form of mercury poisoning based on a comparison of symptoms, as hypothesized by Bernard S, et al. Autism: a novel form of mercury poisoning. *Med. Hypothesis* 2001;56;462-71. The authors, a neuroepidemiologist at the NIH and a neurologist at Harvard Medical School, demonstrate that the Bernard, et al. symptom comparison is simplistic and flawed. Consistent with the Clarkson studies (*see* #1), they state: "At equivalent doses, higher levels of mercury have been found in the blood and less in brain following administration of ethylmercury than methylmercury."

Drs. Nelson and Bauman also note that the pathological differences of brains exposed to methylmercury poisonings have different appearances than those of autistic brains. The most dramatic difference is that brains involved in methylmercury poisonings are smaller than normal, while autistic brains are larger.

The final conclusion expressed by Drs. Nelson and Bauman is: "On the basis of current evidence, we consider it improbable that thimerosal and autism are linked."

Drs. Nelson and Bauman also make the point that there is no environmental conclusion to be drawn from the fact of regression, pointing out that even single gene disorders may have a period of apparently normal development. The dramatic example they cite is Huntington's chorea, where 45 years may pass before the onset of clinically recognizable signs. They state that with autism "the onset of signs in the second year of life does not prove (or disprove) a role for environmental factors in etiology."

**8. "'Whether there is, or is not, any synergistic biological interaction between aluminum and mercury [in vaccine products] is unknown.'"[13]**

The two sentences immediately preceding the one quoted by the Petitioners from the CBER website puts the aluminum issue in context:

> Over a period of 6 months, taking an average weight of 5 kilograms for a child, this [the ATSDR's minimal risk level] would translate into an allowed accumulation of 10.8 milligrams of aluminum. This number is in excess of the 1.5-3.5 milligrams of aluminum that a child would receive from vaccines.

www.fda.gov/cber/vaccine/thimfaq.htm. That being said, however, we have found no published research examining the potential for synergistic interactions between aluminum and mercury.

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[13] *Id.*

**NBTS 34**

**Mercury levels in blood and brain of infant monkeys exposed to thimerosal**

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Recently, questions have arisen about the safety of thimerosal, an ethylmercury containing preservative used in some infant vaccines. Current EPA guidelines for methylmercury exposure (primarily through fish in the diet) have been used to assess the public health risk to children receiving thimerosal-containing immunizations as infants. Depending on the exact vaccinations, schedule, and size of the infant, some children may receive ethylmercury (in the form of thimerosal) approaching or at the EPA guideline levels. The purpose of this study was to compare the distribution of total and inorganic mercury in newborn monkeys following thimerosal exposure (via injection) with newborns exposed to methylmercury (via oral gavage). Infant monkeys were exposed to thimerosal or methylmercury at birth and at 1, 2, and 3 weeks of age. Blood mercury levels were determined 2, 4 and 7 days after each exposure. Brain mercury levels were assessed 2, 4, 7 or 28 days after the last (3 week) exposure. Preliminary data indicate that the half-life of mercury in blood following thimerosal exposure is significantly lower than the half-life of mercury following methylmercury exposure. Brain levels of mercury were also significantly lower in the thimerosal group. Thus far, the data indicate that EPA guidelines for methylmercury exposure may not provide an accurate assessment of the public health risk to children receiving thimerosal-containing immunizations.
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# Human Exposure to Mercury: The Three Modern Dilemmas

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**Key words: methyl mercury; mercury vapor; thimerosal; dental amalgam**

## INTRODUCTION

Humans have been exposed to many different chemical and physical forms of mercury, and all are poisonous. Everyone is exposed to mercury generally at levels that do not elicit overt toxicity. Nevertheless, potential health risks from today's low levels of mercury are the subject of intense debate.

Mercury is the only metal that is liquid at room temperature. Its silvery appearance and mobility as a liquid gained it the name of quicksilver. It is usually prepared by heating the major ore of mercury, cinnabar. This is a red often-crystalline compound of mercuric sulfide.

Quicksilver itself is not toxic. Released from a thermometer accidentally broken in the mouth, it will pass along the gastrointestinal tract to be completely excreted in the feces without any obvious harm to the patient. Indeed, a couple of centuries ago the ingestion of a tablespoonful of quicksilver, and in severe cases 2 pounds in four divided doses, was prescribed for the treatment of constipation [1].

Mercury vapor ($Hg^0$) emitted from its liquid form has caused innumerable cases of human poisonings ever since the time quicksilver was first handled by humans at least two millennia ago [2]. Today quicksilver is still used in thermometers; electric devices, including fluorescent light bulbs; medical equipment, such a blood pressure cuffs; and in the chemical industry. Up to 100 tons of quicksilver are needed as an electrode in a single chlor alkali plant producing chlorine gas and caustic soda from brine.

The difference in the toxicity of ingested metallic mercury and inhaled mercury vapor was emphasized by Ramazzini in his De Morbis Artificum, published in 1713:

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"*We may marvel at the fact that mercury which is well known to be the sole remedy for worms, in fact for killing worms in children nothing is more efficacious, and may be administered harmlessly, either infused in water or decocted or even mixed with some kind of conserve*" while "*its fumes and exhalations are inhaled by the mouth and nose are so deadly that in an instant almost they all but kill the victim..*" [3].

Numerous chemical compounds of mercury have been made over the centuries and found application as medicines, fungicides, and antiseptics. Calomel, mercurous chloride, was used in children's teething powders and in laxatives up to the middle of the 20th century when it was discovered that these practices caused acrodynia [4]. The child with acrodynia had autonomic instability with pink sweaty hands and feet, suffered joint pains and was irritable, asthenic, and photophobic. Only about 1 in 500 children exposed to inorganic mercury develops the disease, which is believed to be the result of a hypersensitivity reaction. The application of organic mercury compounds as fungicides in agriculture, caused many mass outbreaks of poisoning in developing countries. Homemade bread was prepared from seed grain treated with methyl and ethyl mercury fungicides. Inadequate warnings accompanied the treated grain. The use of such forms of mercury has now been internationally banned [5].

Although patterns of human usage have changed over the centuries, occupational exposure still occurs especially to mercury vapor in the mining operations, including gold mining [6], the chlor-alkali industry, and in dentistry [7]. Today the general population is primarily exposed to three different forms of mercury: mercury vapors emitted by dental amalgam fillings, methyl mercury naturally bioaccumulated in fish, and an ethyl mercury compound, thimerosal, which is used as a preservative in certain commonly used childhood vaccines [8].

Each of these three forms of mercury has distinct toxicological characteristics and clinical manifestations. Nevertheless human exposure to these forms of mercury has several characteristics in common: large numbers of individuals are exposed, probably numbering in the billions, no overt clinical cases have been reported, and the extent of actual health risks is debatable. Another common characteristic is that attempts to reduce human exposures may, in themselves, cause health risks. These three modern dilemmas will be the theme of this presentation.

## DENTAL AMALGAM

Dental amalgam by weight 50% mercury along with a number of other metals, mainly silver and copper [7]. It was first introduced into dentistry in France about the middle of the nineteenth century [2]. With a number of subsequent modifications, it has proved to be superior to any other form of tooth filling and is inexpensive.

### History of Human Health Concerns

Concerns have arisen from time to time over possible health risks from its mercury content. Such concerns have erupted into outright confrontations between the antagonists and protagonists over the continued amalgam use. Three major confrontations, usually referred as the "amalgam wars," have so far taken place. The observation that amalgam releases mercury vapor [9] led to the third amalgam war that continues to this day with increasing intensity.

Mercury vapor has long been known as a cause of human poisoning [10]. When inhaled in high amounts, it will produce a triad of characteristic signs and symptoms, namely tremor, gingivitis, and erethism. The latter consists of emotional disturbances, such as excessive shyness and aggression. Behavioral changes have also been elicited in experimental animals [11]. Although occasional high exposures still occur, the concern today is with low-level chronic exposures, such as may be found in certain occupations where mercury is still used. Studies on low-level occupational exposures suggest that mild effects on the nervous system and kidneys may be found at urinary levels at or about 50 μg Hg/g of creatinine, with the possibility of effects at urine levels as low as 25 μg Hg/g creatinine [12]. However, epidemiological studies documenting the 25–50 μg Hg/g of creatinine level are missing.

### Current Health Concerns

Amalgam is the major source of inhaled mercury vapor for the general population because levels in both ambient air and drinking water are insignificant [13]. Urine, blood, and autopsy brain levels correlate with the number of amalgam surfaces (reviewed by WHO, [12]). Kingman et al. [14] have shown that approximately 10 amalgam surfaces will raise urinary concentrations of mercury by 1 μg Hg/L. Thus, amalgam will roughly double urinary mercury levels as compared with amalgam-free samples. These urinary levels are at least an order of magnitude lower than those associated with adverse effects from occupational exposures with one interesting exception.

The prolonged chewing of gum can substantially increase the rate of emission of mercury vapor. Sallsten et al. [15] have shown that urinary concentrations may rise to levels close to occupational safe limits. It was estimated that in Sweden alone over 1000 people might fall into this category. Some may be more susceptible to the toxic effects of mercury as compared with the "healthier worker" for whom occupational limits are set. Chronic nicotine gum chewers are the only subgroup of amalgam bearers so far identified that might face a small risk from the toxic effects of inhaled vapor.

The antagonists in the current amalgam war have taken a different tack in the argument against amalgam. The specter has been raised that long term low-level inhalation of mercury vapor from amalgam might cause or may be an exacerbating factor in the development of such chronic degenerative diseases as Alzheimer's (AD), presenile dementia Parkinson's, and the like. AD has received the most attention because of an early observation that brains of sufferers of this disease had slightly higher mercury levels than controls [16]. Subsequent studies have given mixed support to this finding. Even if it were true, it is possible that the diseased tissue may just take up slightly more mercury than normal tissue. Significantly more work has been done on the role of aluminium in AD, but similarly there is doubt whether aluminium is responsible for AD or its accumulation in neurofibrillary tangles simply depends on a prior happening [17].

Several epidemiological studies have failed to provide any support to a possible connection between amalgam mercury and neurodegenerative disease. These include a long-standing study of 1462 women in Sweden [18], an on-going Swedish adoption/twin study involving some 587 subjects [19], and a study of 129 nuns aged 75 to 102 years [20]. This latter study included eight different tests of cognitive function.

Despite the negative epidemiological findings, both in vitro and in vivo experimental studies continue to raise the possibility that mercury might play some role in the development of AD. Such studies, recently reviewed, [8], raise the possibility that mercury vapor may produce morphological and biochemical changes in nerve cells similar to those seen in Alzheimer's.

### Conclusions

Other than rare cases of contact allergic reactions, so rare that a dentist may not see a single case in his or her professional career, no overt cases of mercury poisoning have been reported over a 150-year period of use. Three epidemiological studies were unable to detect any effects of lifetime exposures on cognition, memory or presenile dementia. Two extensive clinical trials on amalgam are now in progress in the most exhaustive effort to date to detect subtle effects on the nervous system and renal function in children. In the meantime, at least excessive chewing by amalgam bearers should be avoided.

It seems likely that as newer and better dental fillings become available, the use of amalgam will diminish if only for cosmetic reasons. Whether the new filling materials offer less long-term health risk without lowering the dental health is a question still to be resolved. Recent evidence suggests that the replacement composite materials have cytotoxic and genotoxic activities [21,22].

Those of us with mouths well populated with amalgam-filled teeth also face a dilemma. Should we have them removed? Molin et al. [23] have shown that blood mercury levels rise substantially because of exposure to vapor generated by amalgam removal (Fig. 1). In some cases the plasma level may double before slowly declining over about 1 year to a new steady state level about half of the preremoval level. Does this transient peak level of mercury offer a greater risk than long term low level exposure?

## METHYL MERCURY IN FISH

In 2001 The United States Environmental Protection Agency [24] issued a new criteria document in which they announced a new upper safe level (the so-called EPA reference dose) for dietary intake of methyl mercury. The reference dose is five times lower than previous national and international guidelines that have held sway for some 30 years. This action has once more focused attention on health risks from methyl mercury in fish. Fish are the major if not only source of human methyl mercury exposure.

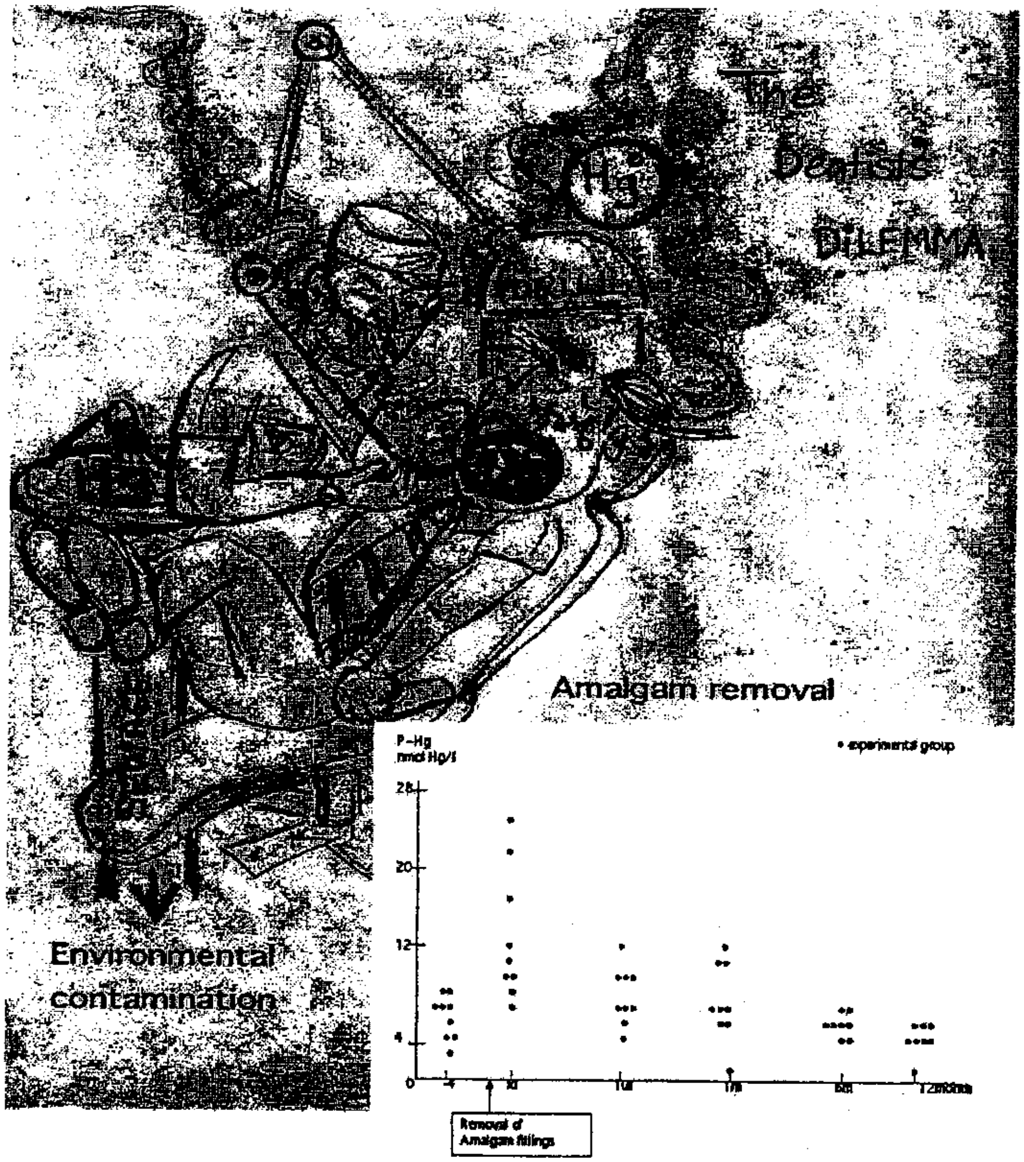


**Fig. 1.** Exposure to mercury vapor from amalgam. Installation of amalgam fillings exposes both dental personnel and patients to mercury vapor. This illustration depicts the removal of dental amalgam by drilling. The amount of vapor generated by this process causes a temporary rise in the patient's blood levels as indicated in the indented graph (adapted from Fig. 1 of Molin et al. 1990 [11]).

The new EPA reference dose is 0.1 μg Hg/kg body weight/day. The regular consumption of only one 7 oz can of tuna per week would supply dietary methyl mercury at a rate close to this reference dose. Although the consumption of certain species of ocean fish with very high mercury levels, such as shark and swordfish, has been the subject of health warnings, this is the first time that the consumption of tuna and other popular species of ocean fish has been questioned. It is therefore an opportune time to look into the toxicology of methyl mercury and into the scientific basis of this dramatic reduction in permissible intake.

## History of Human Exposure

The first alkyl (methyl and ethyl) mercury compounds were synthesized in a chemical laboratory in London in the 1860s [10]. Two of the laboratory technicians involved in the synthesis were fatally poisoned. This so shocked the chemical community that alkyl mercury compounds were given a wide margin for the rest of the century. However, early in the 20th century the potent antifungal properties of the short chain alkyl mercury compounds were discovered, leading to their application to seed grain, especially for cereal crops. The widespread global use of these mercury compounds was found to be highly protective of what otherwise would be devastating fungal infections and the loss of the grain harvest.

Despite this widespread use, few cases of poisoning were reported for the first half of the 20th century. However, in the late fifties and early sixties, serious outbreaks of alkyl mercury poisoning erupted in several developing countries [25]. These outbreaks were caused by individuals preparing homemade bread directly from the treated seed grain. The largest, most recent outbreak took place in rural Iraq in the winter of 1971–1972 [26]. Some 6000 cases were admitted to hospitals. An epidemiological follow-up suggested that as many as 40,000 individuals may have been poisoned [27].

In the late fifties, evidence emerged of environmental damage from mercury treated grain. It was observed in Sweden that predatory birds were developing neurological disorders. These birds were on the top of a food chain starting with small mammals consuming the treated grain freshly planted in the fields. Analysis of feathers from museum-preserved bird specimens indicated a sharp rise in mercury levels at the date when mercurial compounds were first introduced as agricultural fungicides. Because some of these birds were migratory, it was possible to show that elevated mercury levels were found only in those feathers that grew when the birds were in Sweden.

As a control measure, the Swedish investigators decided to check mercury levels in the feathers of fish-eating birds. It was assumed that mercury level should be low. To their astonishment, mercury levels were elevated despite the fact that these birds had no dietary connection with the treated grain (Fig. 2). The mercury levels in feathers taken from museum specimens exhibited a gradual but accelerating rise over a period corresponding to the growth of industrialization in Sweden.

**Fig. 2.** Exposure to mercury from fish. The global cycle of mercury. Mercury vapor, a stable monatomic gas, evaporates from the earth's surface (both soil and water) and is emitted by volcanoes. Anthropogenic sources to the atmosphere include emissions from coal burning power stations and municipal incinerators. Mercury vapor has a residence time of about one year before it is converted to a soluble form and returned in rainwater. It may be converted back to the vapor form both in soil and in water by microorganisms and re-emitted to the atmosphere. Thus mercury may re-circulate for long periods of time. Mercury attached to aquatic sediments is subject to microbial conversion to methyl mercury whereupon it enters an aquatic food chain. It is avidly bioaccumulated to reach its highest concentrations in long-lived predatory fish. The indented figure on the right hand side indicates the routes of transformation to methylmercury as originally suggested by Jernelov [28]. The indented graph on the left depicts the increase in mercury in feathers of fish-eating birds in Sweden (From Figs. 10–6 in Johnels and Westermark [29].) The period of time covered by these data corresponds approximately to the growth of industrialization in Sweden.

Eventually this finding led to a landmark discovery on the environmental fate of mercury, namely that micro-organisms in the aquatic sediments are capable of converting inorganic to methyl mercury [28,29]. This is the first step in its entry into our diet. Methyl mercury bioaccumulates in the aquatic food chain, from plankton to herbivorous fish and finally to the top fish predators, such as shark, swordfish, and to fish-eating marine mammals. A similar food chain exists in bodies of fresh water with such species as pike and bass having some of the highest levels.

The potential for bioaccumulation in aquatic food chains was dramatically demonstrated in two outbreaks of human poisoning in Japan. The Japanese health authorities in Minamata had been aware for some time that fishermen and their families were suffering from a neurological disease exhibiting signs of incoordination, constricted visual fields, and numbness in the extremities. The cause was elusive until a visiting physician from Scotland recognized that the neurological signs and symptoms were similar to cases of occupational methyl mercury poisoning he had seen in England in 1939 [30]. Eventually, the source was traced to a factory manufacturing acetaldehyde where an inorganic compound of mercury was used as a catalyst. The producers were unaware that the synthetic process converted some of the mercury to methyl mercury. Consequently, both methyl and inorganic mercury were discharged into Minamata Bay. It was difficult to believe that methyl mercury released into a large ocean bay could be bioaccumulated to such an extent that the fish carried levels of methyl mercury that would prove lethal when consumed by humans. However, the amount of mercury discharged from 1932 to 1968 was large and estimated at 456 tons of total mercury [31] and about one ton of methyl mercury [32].

The twin discoveries of biomethylation and bioaccumulation aroused intense interest in the environmental fate of mercury and in pathways to human exposure. Methyl mercury was soon detected in all species of fish and in fish-consuming animals. The source appeared to be inorganic mercury biomethylated by micro-organisms in sediments of both fresh and ocean water. Many anthropogenic sources were identified. Chlor-alkali plants discharged inorganic mercury as waste into rivers, lakes and ocean bays. Paper pulp factories likewise discharged a variety of mercury compounds used as slimicides.

Many of these practices have been eliminated but atmospheric contamination still occurs world-wide because of fossil fuel combustion. Smelting, cement production, and refuse incineration add to the atmospheric release of mercury [7]. Contamination of water and air occurs from extensive gold "mining operations, for example, in the Amazon basin [6]. Large quantities of liquid mercury are used to extract the sedimentary gold found in riverbeds. Pure gold is recovered when the mercury is evaporated from the amalgam by heating. It has been estimated that over 130 tons of mercury has been released each year to the Amazon basin alone [33].

**The global cycling of mercury.** The global cycling of mercury (Fig. 2) begins with the evaporation of mercury vapor from land and sea surfaces. Volcanoes can be an important natural source [34]. The burning of fossil fuel, especially coal and municipal waste incineration, are major anthropogenic sources adding to the atmosphere. Mercury vapor is a chemically stable monatomic gas. Its residence time in the general atmosphere is estimated to be about 1 year. Thus, mercury vapor is globally distributed even from point sources. By processes not yet fully understood, the vapor is oxidized in the upper atmosphere to a water-soluble ionic mercury, which is returned to the earth's surface in rainwater. Some of the mercury in rainfall reaches the aquatic environment, mainly the oceans. About 90% of the total Hg input to oceans is recycled to the atmosphere and less then 10% reaches the sediments. However, 2% is methylated in the biota resulting in accumulation in the food chain. Only a small fraction is lost to the atmosphere, mainly as highly volatile dimethyl mercury [35]. The global cycling of mercury results in the distribution of mercury to the most remote regions of the planet. For example, environmental mercury levels even in arctic water are similar to those in more southern latitudes [36].

**Absorption, disposition, and excretion.** Several recent reviews have given extensive details on the disposition of methyl mercury in the body [7,24]. A brief review and update will be given here. About 95% of methyl mercury ingested in fish is absorbed in the gastrointestinal tract. The exact site of absorption is not known. It is distributed to all tissues, the process being complete in about 30 h. About 5% is found in the blood compartment and about 10% in brain. The concentration in red blood cells is about 20 times the concentration in plasma. It crosses the blood–brain and placental barriers. Levels in cord blood are proportional to but slightly higher than levels in maternal blood. Levels in the fetal brain are about five to seven times higher than levels in maternal blood [37]. Brain-to-blood ratios in adult humans and other primates are approximately in the same range.

Methyl mercury avidly accumulates in growing scalp hair. Concentrations in hair are proportional to simultaneous concentrations in blood but are about 250 times higher. They are also proportional to concentrations in the target tissue, the brain [37]. Longitudinal analysis of strands of scalp hair can recapitulate past blood levels [38]. Hair and blood are used as biological indicator media of methyl mercury exposure in both the adult and fetal brain (in the latter case, maternal hair or cord blood).

Methyl mercury is slowly metabolized to inorganic mercury by microflora in the intestines and by phagocytic cells. The biochemical mechanisms are unknown. Although methyl mercury is the predominant form of mercury during exposure, inorganic mercury slowly accumulates and resides for long periods in the central nervous system. It is believed to be in an inert form probably in the form of insoluble mercury selenide (see below).

Urinary excretion is negligible, of the order of 10% or less of total elimination from the body. Methyl mercury undergoes extensive enterohepatic cycling [39]. It is secreted into bile and partly reabsorbed back into the portal circulation and thereby returned to the liver. A fraction of the biliary mercury is converted by microflora to inorganic mercury. The latter is reabsorbed only to a small extent. Thus, most of the methyl mercury is eliminated from the body by demethylation and excretion of the inorganic form in the feces. The processes of biliary excretion and demethylation by microflora do not occur in suckling animals. The role of these two processes in suckling human infants is unknown.

The high mobility of methyl mercury in the body is not the result of lipid solubility as claimed in some textbooks. Methyl mercury is present in the body as water-soluble complexes mainly, if not exclusively, attached to the sulfur atom of thiol ligands. It enters the endothelial cells of the blood-brain barrier as a complex with L-cysteine. The process is so specific that the complex with the optical isomer, D-cysteine is not transported. Structurally the L-complex is similar to the large neutral amino acid, L-methionine and is carried across the cell membrane on the large neutral amino acid carrier [40].

Methyl mercury is pumped out of mammalian cells as a complex with reduced glutathione. For example, it is secreted into bile as a glutathione complex. The glutathione moiety is degraded in the bile ducts and gall bladder to a dipeptide and finally to the L-cysteine complex. Presumably in this form it is reabsorbed back into the blood stream to be returned to the liver thereby completing the enterohepatic cycle [41,42].

The elimination of methyl mercury from the body approximately follows first order kinetics. Half times vary from one tissue to another, but generally fall in the range of 50 to 70 days when measured as total mercury. However, when measured as methyl mercury the half time in blood is lower, about 44 days taking into account conversion to inorganic mercury [43]. Thus individuals with long term regular exposure to methyl mercury attain a steady state body burden in about one year (five half times).

**Adverse effects.** The major toxic effects of methyl mercury are on the central nervous system. Its toxic action on the developing brain differs in both mechanism and outcome from its action on the mature organ so the two actions will be treated separately (for a detailed review, ref [7]).

**The mature central nervous system.** Methyl mercury toxicity in adults is characterized by a latent period between exposure and the onset of symptoms [26,44]. The period can be several weeks or even months depending on the dose and exposure period. A dramatic example of latency was the case of severe, ultimately fatal poisoning of a chemistry professor from exposure to dimethyl mercury [45]. A single exposure caused by a small spill of the liquid dimethyl mercury took place in the month of August. The professor continued her normal professional work without any apparent ill effects. In November she presented a paper at an overseas conference. It was not until late December that the first symptoms appeared. Within a few weeks the full syndrome of severe methyl mercury poisoning became manifest. Despite many decades of research on methyl mercury toxicology, the mechanism underlying this long latent period in still unknown.

Paresthesias, a numbness or a "pins and needles" sensation, is the first symptom to appear at the lowest dose [26]. This may progress to cerebellar ataxia, dysarthria, constriction of the visual fields, and loss of hearing. These signs and symptoms are caused by a loss of neuronal cells in specific anatomical regions of the brain. For example, ataxia results from the loss of the granules cells in the cerebellum. The neighboring Purkinje cells are relatively unaffected.

The mechanism underlying the focal damage to the adult brain is still not established with any certainty. Syversen [46] examined the effect on protein synthesis in different areas of the brain of rats poisoned with methyl mercury. Protein synthesis was inhibited in all three areas studied, the granule and Purkinje cells of the cerebellum, and cells from the cortical areas of the brain. However, protein synthesis recovered in two types of neurons but not in the granule cells. These data suggest that focal damage in the brain depends on interplay between initial insult and repair. Jacobs et al. [47] pointed to the importance of the repair process in determining selective damage. Apparently the small granule cells lack the repair systems present in the other larger cells. Sarafian et al. [48] have suggested that the selective vulnerability of cells in the nervous system may arise from a "critical absence of inherent protective mechanisms."

Cellular defenses may be decisive in determining the toxic outcome and deserve further study. Thiol compounds probably play a key role. Resistant cells have higher levels of the thiol containing peptide, glutathione [49]. Glutathione also plays a key role in the excretion of methyl mercury (for further discussion, see ref [48]).

Methyl mercury is converted to inorganic mercury in the brain and autopsy samples taken years after exposure to methyl mercury reveal that inorganic species account for most if not all the remaining mercury in the brain [50]. It has been suggested that the long residence time is due to inorganic mercury forming an insoluble complex with selenium [13]. Moreover, Charleston et al. [51] observed that in the thalamus of monkey, methyl mercury concentration plateaus at around 12 months exposure while inorganic levels of mercury increased even after the cessation of exposure to methyl mercury. Based on this observation, the authors suggested that inorganic mercury may be a proximate toxic form. However, experiments comparing methyl and ethyl mercury compounds on rats suggest that the intact methyl mercury is the toxic form. Ethyl mercury, even in the brain, converts to inorganic mercury more rapidly than methyl mercury but the latter produced more severe damage [52].

**Effects on the developing brain.** The first indication of the special susceptibility of the developing brain to prenatal exposure to methyl mercury came from the outbreak of poisoning in Minamata. Mothers with mild symptoms gave birth to offspring with severe brain damage [53]. The Iraq outbreak confirmed that severe brain damage can occur from high prenatal exposure. A milder syndrome was also identified in the Iraq outbreak [54]. Children, apparently normal, nevertheless had a history of delayed achievement of developmental milestones and, on examination, exhibited mild neurological abnormalities such as brisk tendon reflexes. When prenatal exposure was determined from mercury levels in maternal hair samples, it was possible to construct a dose-response relationship between peak hair mercury levels in pregnancy and functional developmental delays in walking and talking and abnormal neurological findings (Fig. 2) [55].

This study was of interest for two reasons. It was the first time that a dose/response relationship had been established between a dose measured in one individual, namely, the mother and effects observed in another, namely, her offspring. It laid the groundwork for further quantitative estimate of prenatal risks from methyl mercury. This relationship was made possible by the parallel between levels of mercury in maternal and fetal tissues. Indeed it was later demonstrated in another study that maternal hair levels of mercury were proportional to levels in autopsy samples of brain tissue from infants dying shortly after birth [37].

Another unique aspect of the Iraq study was the application of continuous single strand hair analysis to determine peak levels during pregnancy. By the use of X-ray fluorescent analysis, it was possible to measure the concentration of mercury in contiguous 2-millimeter segments of a single strand of maternal hair thus giving a complete picture of mercury exposure levels during pregnancy. Moreover, because exposure in Iraq took place over a single short period of time, it was possible to fit the hair data with a single compartment model covering both the rising levels during intake and the exponential fall afterwards. This allowed the true peak value to be calculated from the curve by fitting all the data points as opposed to taking the single highest value that would be more prone to error.

The studies of the Iraq outbreak confirmed what had been suspected from the outbreak in Japan, namely, that the fetal developing brain was more sensitive than the mature organ. Earlier, a Swedish expert group [56] had estimated a threshold level for effects in adults at about 50 ppm in hair, an estimate confirmed by the findings in Iraq [26]. This level may be compared to an estimated threshold as low as 10 ppm for prenatal effects based on Iraq data [55].

In the two outbreaks of methyl mercury poisoning in Japan, there was only one congenital case of methyl mercury poisoning where the exposure is specifically known. In Minamata, no specific exposures of congenital patients were known. However, in Niigata, the poisoning was recognized early and pregnant mothers with hair levels above 50 ppm were offered abortions [57]. There were 13 mothers who chose to have their babies. One mother with a hair level of 293 ppm delivered a child with a neurological handicap believed to be congenital poisoning [58]. The other 12 mothers had hair mercury levels ranging from 51 to 115 ppm. Their children were examined at age 5 years by a pediatrician and said to be normal.

As these studies were being conducted and early findings presented at scientific meetings, concern arose that methyl mercury in fish normally consumed in our diet might present risks of prenatal damage. Several large epidemiological studies were conducted in people consuming freshwater [59] and ocean water fish [60,61]. Large scale studies are continuing to this day [62,63]. These studies have not yet provided a consistent picture of the lowest prenatal levels that offer a measurable risk of damage to the developing brain. However, at this time it can be said that these studies on fish eating populations taken as a whole are still consistent with the original findings from Iraq that effects may occur in the exposure range of 10–20 ppm in maternal hair growing during pregnancy.

**Mechanism of prenatal damage.** Several studies have given some insight into the mechanism underlying prenatal brain damage. Autopsy brain samples from the Minamata outbreak indicated a widespread damage to all areas of the fetal brain as opposed to the focal lesions seen in adult tissue. Microcephally was also observed [13]. Autopsy tissue from Iraq also gave invaluable clues to the nature of prenatal brain damage [64]. The normally ordered parallel arrays of neuronal cells in the cortex were found to be disrupted indicative of a general disturbance in neuronal migration and lamination. Moreover, neurons were present such as Purkinje cells that had failed to migrate to the cerebellum. These findings from both Japan and Iraq indicated that the most basic processes in brain development were affected, namely neuronal cell division, migration and organization.

Experimental work in animals and in vitro has provided mechanisms explaining why methyl mercury inhibits both cell division and migration [65–67]. The studies show that the cytoarchitecture first affected at the lowest levels of methyl mercury is the microtubular system. Intact microtubules are required for both cell division and migration. Microtubules are formed by a treadmilling process whereby assembly from alpha and beta tubulin monomers takes place at one end and disassembly at the other. Apparently methyl mercury blocks the assembly process, but the disassembly continues unchanged thus leading to the complete loss of the microtubule.

### Conclusions

A number of epidemiological studies have indicated that the developing brain, especially during the prenatal stage, is the most susceptible tissue for the toxic action of methyl mercury in humans. Experimental studies have revealed mechanisms for the efficient transport to and toxic action on the developing brain. Nevertheless, the degree of risk in the general population is still speculative with the weight of evidence suggesting a maternal hair level during pregnancy of 10 ppm or greater is needed. The general population in most countries averages about 1 ppm although heavy fish consumers may approach or exceed 10 ppm. At issue is the magnitude of the safety factor that should be applied.

In contrast, the health benefits of fish in the diet are well established. The public health dilemma is whether or not to restrict fish consumption based on the rather tenuous evidence that levels of methyl mercury currently found in fish consumers may present a risk to the developing brain. Currently certain national guidelines identify species of fish with the highest mercury levels as unfit for consumption. Generally such species have average methyl mercury in edible tissues exceeding 1 ppm. The dilemma now facing regulatory authorities is to what extent restrictions should be extended to lower levels in fish, thereby affecting the consumption of popular species of fish by the general population.

## THIMEROSAL AND ETHYL MERCURY COMPOUNDS

In the late summer of 1999, concern was expressed by a major professional pediatric society (the American Academy of Pediatrics) and by the Public Health Service of the USA about the safety of a mercury preservative in many vaccine preparations routinely administered to infants [68]. These concerns arose directly from a report by Ball et al. [69], to be discussed below, that the routine administration of vaccines to infants in the first 6 months of life could exceed USEPA guidelines for safe intakes of mercury and might be related to autism in children. Within a period of 18 months or so, the mercury preservative had been removed by the manufacturers from all vaccines destined for use in the United States.

The mercury preservative has the trade name Thimerosal and has the molecular formula: $CH_3CH_2$–S-$C_6H_4$-COOH. This preservative was introduced into vaccines in the early 1930s and has been used ever since [70]. It was given a clean bill of health by the US FDA in 1976 [69]. In the meantime, the US EPA had lowered its allowable safe long-term daily intake for mercury, usually referred to as the EPA reference dose. As a result, in a more recent review of Thimerosal by the USFDA, questions were raised about possible health risks.

Mercury in Thimerosal is in the form of ethyl mercury ($CH_3CH_2$-$Hg^+$) for which there is limited toxicological information. Estimates of health risks from Thimerosal were based on the assumption that ethyl mercury was toxicologically similar to its close chemical relative methyl mercury ($CH_3$-$Hg^+$) about which a great deal is known. Thus the latest USEPA guidelines for methyl mercury were used.

The concern over health risks from Thimerosal had other consequences. The administration of certain vaccines was delayed to a later age [71], and groups long opposed to the use of vaccines now had new ammunition. Autism is usually first diagnosed in infants about the time of their last vaccination, leading to an apparent temporal relationship. Thimerosal attracted attention as the active agent in vaccines that might be a potential cause [72]. As a result, there is now intense interest in the toxicological properties of ethyl mercury especially as administered to infants in the compound Thimerosal.

## History of Human Poisonings

Ethyl mercury compounds were first synthesized in the 19th century in a chemical laboratory in London [10]. One of these compounds, diethyl mercury, is highly volatile and readily absorbed in the lungs. As a result of this first synthesis, the unsuspecting chemist inhaled a fatal dose. The publicity associated with this poisoning served to curtail commercial application of ethyl and the closely related methyl mercury compounds. However, early in the 20th century, the fungicidal properties of these short chain alkyl mercury compounds led to commercial applications in agriculture. For example, they are especially effective in a plant root disease in wheat caused by *Telletia caries* and *Telletia foetiida* [73]. In fact many different organic mercury compounds were being used to prevent seed borne diseases of cereal by 1914 [74].

Generally speaking, the ethyl mercury fungicides were used effectively and safely. However, a number of outbreaks of poisoning occurred in some developing countries [10]. For example, two outbreaks took place in rural Iraq in 1956 and 1960 from the misuse of the fungicide, ethyl mercury toluene sulphonilamide [74]. The farmers' families prepared homemade bread directly from the treated grain instead of planting it. Hundreds of cases of severe poisoning occurred, many of which had a fatal outcome. Cases of ethyl mercury poisoning have also occurred in China as recently as the 1970s. The exposure pathway was the same as in Iraq. The farmers consumed rice treated with ethyl mercury chloride [75].

Ethyl mercury in the form of Thimerosal has found wide application in medicine as a disinfectant. Axton [76] reported case histories of four children and two adults severely poisoned by accidental exposure. Five of the six cases died. Rohyans et al. [77] reported a case of severe poisoning from treatment of an infected ear. Pfab et al. [78] reported on an attempted suicide from drinking a solution of Thimerosal resulting in severe poisoning. The treatment of infants with omphaloceles resulted in high levels of mercury in autopsy tissues [79]. The only known cases of acrodynia from ethyl mercury was reported by Matheson et al. [80] in a patient with long term injection of a gamma globulin solution containing Thimerosal. Acrodynia is mainly a childhood disease that has largely disappeared due to the discovery in the 1950's of mercury as its cause [4].

The cases discussed above have been the result of high doses of ethyl mercury compounds. Lower doses have been associated with rare cases of allergic response. Its use as a skin disinfectant has led to cases of contact dermatitis [81,82].

Since the application of ethyl mercury as a disinfectant in vaccines in the 1930s, no clinical cases of poisoning by this route have ever been reported despite the fact that billions of infants must have been exposed from this source. However, cases of hypersensitivity have been reported from its use in vaccines [83,84].

## Current Health Concerns

The estimates of health risk from Thimerosal were based on the application of the USEPA reference dose [69]. This reference dose was derived from data in the lowest observed prenatal exposure to methyl mercury that gave rise to adverse developmental effects in the offspring in the outbreak of methyl mercury poisoning in Iraq [85] and, subsequently, on neuropsychological tests on children exposed prenatally to methyl mercury in whale meat [86]. The fact that vaccination results only in postnatal exposures must shed some doubt on validity of the application of this guideline. The assumption that the toxicological properties of ethyl mercury are identical to those of methyl mercury is also questionable.

**Disposition of ethyl versus methyl mercury.** Matheson at al. [80] reported on blood and urine levels in one patient exposed to Thimerosal in long-term injections of gamma globulin. Specifically, they reported on levels of mercury before and after one injection of gamma globulin. The data allow a rough calculation to see if the observed increase in blood level is similar to that expected from methyl mercury. The injected dose was 0.6 ml/kg containing 50.3 μg Hg/ml to give a total mercury dose of 30 μg Hg/kg. The disposition parameters for methyl mercury in adult humans [13] predict that 5% of the dose, 1.5 μg Hg/kg, is deposited in the blood compartment. The volume of the latter is 70 mL/kg, assuming the blood compartment is 7% of the body weight. Thus 1.5 μg Hg/kg would be deposited in 70 mL of blood per kilogram body weight to give an increase on concentration of $1.5 \times 1000/70$ μg Hg/liter = 21 μg Hg/L. The observed increase was 18 μg Hg/L. This calculation would suggest that the disposition of mercury after Thimerosal is not very different from that expected from methyl mercury.

Stajich et al. [87] were the first authors to measure disposition of mercury before and after administration of vaccines containing Thimerosal. They reported on blood levels of mercury before and 48 to 72 h after administration of a single dose of hepatitis vaccine in the first week of life. Seven were preterm infants with an average birth weight of 748 g and five were term infants with an average birth weight of3588 g. Their prevaccination blood levels ranged from

0.04–0.5 μg Hg/L. The preterm levels rose to an average value of 7.4 μg Hg/L whereas the term infants attained 2.2 μg Hg/L (Fig. 3).

The question arises whether these levels are similar to those expected from methyl mercury. The dose expressed as μg Hg/kg body weight was much higher in the preterm infants (16.7 μg Hg/kg for preterm versus 3.5 μg Hg/kg for full term infants) but the absolute dose was the same for all infants, 12.5 μg Hg. It is assuming that 5% or 0.625 μg Hg should be deposited in the blood compartment and that is assumed to be 8% of the infant's body weight. Thus, for the preterm infants, 0.625 μg Hg would be deposited in a blood volume of $.08 \times 748 = 60$ ml to give a predicted concentration of $0.625 \times 1000/60 = 10.4$ μg Hg/L. This compares to an observed increase of 6.8 μg Hg/L. The predicted increase for the term infants based on methyl mercury is $0.625 \times 1000/287 = 2.2$. The observed increase was identical, 2.2 μg Hg/L.

These estimates also suggest that the disposition of mercury after a dose of Thimerosal is similar to that expected from methyl mercury. However these estimates can be regarded as approximate at best. Individual values for each infant were not reported. The blood levels in samples collected between 48 to 72 h may not have been the true maximum levels after distribution of the injected dose.

A recent report by Pichichero et al. [88] indicate blood levels of mercury in infants lower than what would be expected from methyl mercury. These infants, aged up to 6 months, had received vaccines some of which contained Thimerosal. Most blood samples were collected one week or more after the last vaccination. The highest recorded level was 4.1 μg Hg/L and many were below the detection limit of about 0.5 μg Hg/L. The authors calculated that the half time in blood was approximately 7 days, a value considerably smaller than that generally accepted for methyl mercury of about 50 days [13].

The main difference in design from the Stajich et al. study is that samples were collected much later after the last dose of Thimerosal. Both studies could be consistent if ethyl mercury is excreted more rapidly than methyl mercury. The time after collection of 48 to 72 h is too short for a measurable effect of excretion on blood levels in the Stajich et al. study.

In conclusion, it would appear that the initial tissue distribution of ethyl mercury is similar to that of methyl mercury. However, the residence time in the body appears to be much shorter such that the potential for accumulation of mercury after repeated vaccinations in the first six months of life would appear to be much less than that expected from methyl mercury. A half time of only 7 days as reported by Pichichero et al. [88] would indicate that ethyl mercury should have virtually disappeared from infant tissues in the usual two month interval between vaccinations.

**Toxic effects of ethyl versus methyl mercury.** Magos et al. [52] compared the tissue distribution and toxicity of ethyl versus methyl mercury in rats. Five daily doses of 8 mg hg/kg were given by gavage for five consecutive days. The difference in the brain damage between methyl- and ethyl mercury-treated rats demonstrated the importance of the blood–brain barrier against ethyl mercury. Ten days after the last treatment with equal doses of 10 mg Hg/kg, only methyl mercury treated rats had damage in the granular layer of the cerebellum. No damage was observed in ethyl mercury treated rats. Even a 20% increase in the

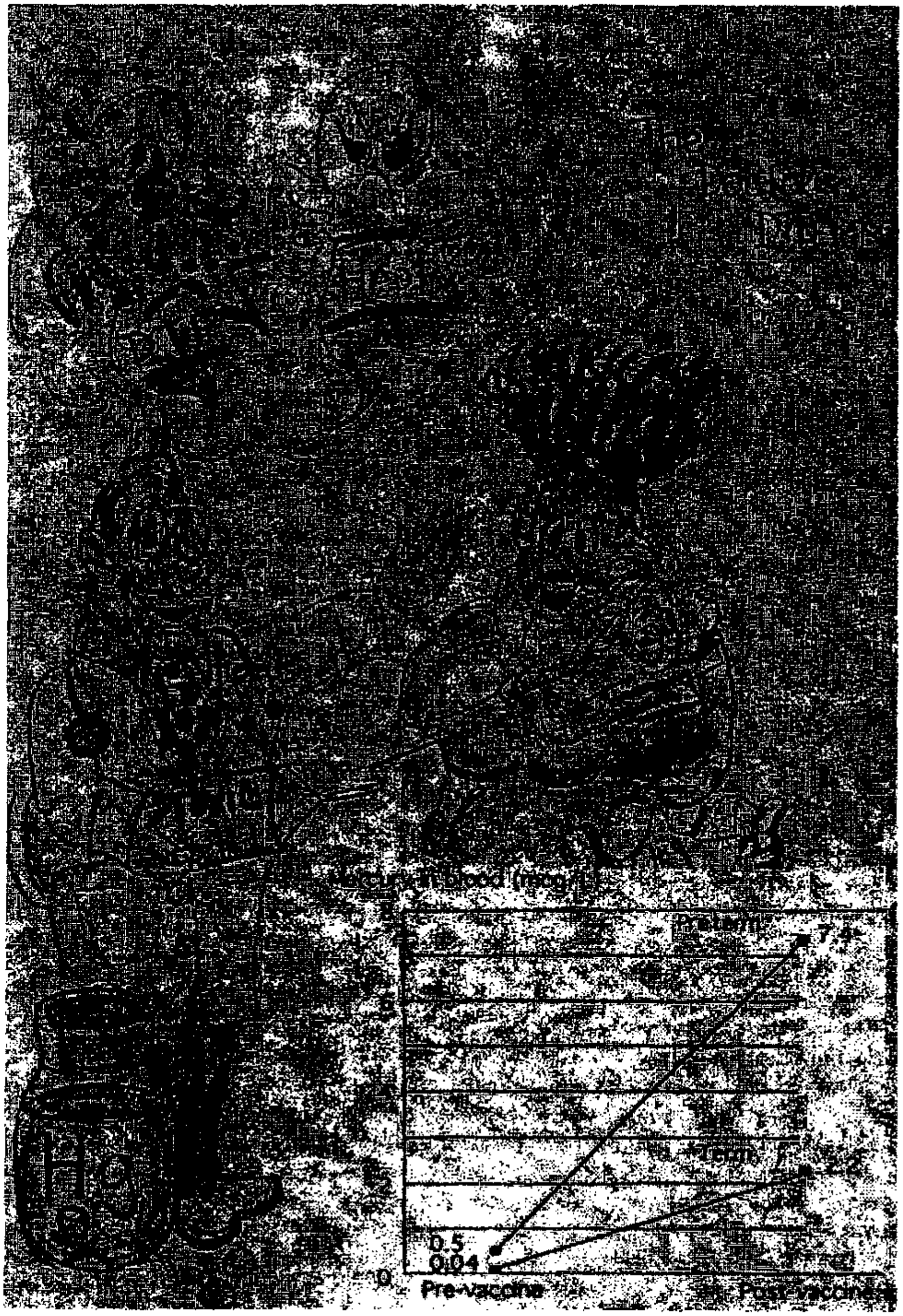


Fig. 3. Exposure to thimerosal in vaccines. The news media and some authors have claimed that thimerosal in vaccines poses a health risks to infants. The indented graph depicts the rise in blood concentration in newborns receiving a single shot of hepatitis B vaccine containing 12.5 μg of mercury (adapted from Fig. 1 of Stajich et al. [87]). The infants of normal body weight experience a rise of only 2 ppb in blood, well below toxic levels. However, premature infants of lower body weight can develop blood levels just in excess of the US EPA reference dose.

dose (which is near to the lethal dose) of ethyl mercury caused less damage than seen in methyl mercury treated rats. Similar protective mechanisms were not seen with respect to body weight, kidney, or spinal root ganglia.

Magos [89] has reviewed the published cases of human poisoning resulting from exposure to Thimerosal. Severe cases of poisoning can result in the same neurological signs and symptoms associated with methyl mercury poisoning, for example, constriction of the visual fields. Ethyl mercury poisoning was characterized by a latent period of several weeks between first exposure and onset of the first symptom of poisoning as has been observed for methyl mercury. In distinction from methyl mercury, signs of renal damage were found in severe poisoning cases.

A detailed review of case histories on exposure to ethyl mercury including Thimerosal, allowed Magos to construct a table comparing blood levels at the time of onset of symptoms. To estimate such blood levels from samples collected at a later date, he assumed a half time in blood of 50 days. Severe intoxication was associated with blood levels in excess of 2000 μg Hg/L with milder intoxication at 1000 mg Hg/L. Five cases with blood levels in the range of 140 to 650 μg Hg/L had no reported adverse effects. Because of the small number of individuals, 18 altogether, no statistical evaluation was possible in terms of dose response relationships. However, the data suggest that ethyl mercury is somewhat less potent in producing neurological signs and symptoms as compared to methyl mercury where the threshold for adverse effects has been estimated at about 200 μg Hg/L [13].

Allergic responses, usually by skin application, are well known to occur from organo-mercurial compounds including Thimerosal [90]. Santucci et al. [91] have demonstrated that contact allergy to Thimerosal is caused by the ethyl mercury radical and that it is indistinguishable in its allergic reaction from methyl mercury. Goncalo et al. [92] also noted that allergy to Thimerosal was mainly related to the mercurial component. No allergy was seen to the intact Thimerosal molecule although some allergic reactions may be the result of the thiosalicylic acid component.

Allergy to Thimerosal and related mercury compounds does not develop in all exposed individuals. There is evidence that individuals with certain polymorphisms in glutathione transferase genes may be more susceptible to allergic reactions to Thimerosal. However, glutathione is necessary for the biliary excretion of methyl and inorganic mercury [39] and intracellular glutathione is protective against the toxicity of methyl mercury [49].

Only one case of acrodynia has been reported from exposure to Thimerosal [80]. This occurred in a 20-year-old man receiving regular gamma globulin infusions containing Thimerosal as a preservative. The total dose was estimated to be 40 to 50 mg Hg.

## Conclusions on Health Risks

Ethyl mercury and therefore thimerosal would appear to be less toxic to humans than methyl mercury compounds. The primary reason is that the potential for accumulation from repeated exposure may be lower. Thus estimates of health risks based on the US EPA reference dose may have over estimated the risks. However some caution is in order since ethyl mercury produces at least two toxic effects that methyl mercury does not, namely kidney damage and possibly acrodynia. The dilemma faced by the physician today (Fig. 3) is the known and indeed necessary public health benefits of vaccination versus essentially hypothetical risk estimates of brain and kidney damage from ethyl mercury. The USA public health authorities have already decided to retire Thimerosal from its 70 years of active service and now rely mainly on single use vials for vaccines that require no preservative. The World Health Organization [93] has taken a different view as the need for multiple use vaccine vials has overwhelming advantages if not absolute needs in developing countries.

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## REFERENCES

1. Polson CJ, Tattersall RN. Clinical toxicology. London: The English University Press; 1959.
2. Goldwater LJ. Mercury: a history of quicksilver. Baltimore: York Press; 1972.
3. Ramazzini B (1713) Diseases of Workers. (Translated by Wilmer Cave Wright) New York: Hafner Publishing. Company; 1964. p 42.
4. Warkany J, Hubbard DM. Acrodynia and mercury. J Pediatr 1953;42:365–386.
5. FAO-WHO. Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth report of the joint FAO-WHO Expert Committee on Food Additives. World Health organization Technical Report Series. 1974;539:1–40.
6. Cleary D. Anatomy of the amazon gold rush. Iowa City, Iowa: University of Iowa Press; 1990.
7. ATSDR. Toxicological profile for mercury. Agency for Toxic substances and disease. Atlanta: Division of Toxicology, US Department of Health and Human Services, Public Health Service; 1999.
8. Clarkson TW. The three modern faces of mercury. Environ Health Perspect 2002;110(suppl 1):11–23.
9. Frykholm KO. On mercury from dental amalgam, its toxic and allergic effects. Acta Odontol Scand 1957; 5 (suppl 22):17.
10. Hunter D. Diseases of occupations. Boston: Little Brown & Co; 1969. p 314–328.
11. Newland MC, Warfinge K, Berlin M. Behavioral consequences of in utero exposure to mercury vapor: alterations in lever-press durations and learning in squirrel monkeys. Toxicol Appl Pharmacol 1996;139:374–386.
12. WHO. Environmental Health Criteria 118. Inorganic Mercury. International program on Chemical Safety. Geneva: World Health Organization; 1991.
13. WHO. Environmental Health Criteria 101: Methyl mercury. International Program on Chemical Safety, Geneva: World Health Organization; 1990.
14. Kingman A, Albertini T, Brown LJ. Mercury concentrations in urine and whole blood associated with amalgam exposure in a US military population. J Dent Res 1998;77:461–467.
15. Sallsten G, Thoren J, Barregard L, Schutz A, Skarping G. Long-term use of nicotine chewing gum and mercury exposure from dental amalgam fillings. J Dent Res 1996;75:594–598.
16. Thompson CM, Markesbery WR, Ehmann WD, Mao YX, Vance DE. Regional brain trace-element studies in Alzheimer's disease. Neurotoxicology 1998;9:1–7.
17. Chadwick DJ, Whelan J. Aluminium in Biology and Medicine. Ciba Foundation Symposium 169. Chichester: John Wiley & Sons; 1992.

18. Ahlqwist M, Bengtsson C, Laipus L, Gergdahl IA, Schutz A. Serum mercury concentration in relation to survival, symptoms and disease: results from a prospective population study of women in Gothenburg, Sweden. Acta Odontl Scand 1999;57:168–174.
19. Bjorkman L, Pedersen NNL, Lichtenstein P. Physical and mental health related to dental amalgam fillings in Swedish twins. Comm Dent Oral Epidemiol 1996;24:260–267.
20. Saxe SR, Snowden DA, Wekstein MW, Henry RG, Grant FT, Donegan SJ, Wekstein DR. Dental amalgam and cognitive function in older women: findings from the Nun Study. J Am Dent Assoc 1995;126:1495–1501.
21. Reichl FX, Walther U, Durner J, Kehe K, Hickel R, Kunzelmann KH, Spahl W, Hume WR, Benschop H, Forth W. Cytotoxicity of dental composite components and mercury compounds in lung cells. Dent Mater 2001;17:95–101.
22. Heil J, Reifferscheid G, Waldmann P, Leyhausen G, Geurtsen W. Genotoxicity of dental materials. Mutat Res 1996;368:181–194.
23. Molin M, Bergman B, Marklund SL, Schutz A, Skerfving S. Mercury, selenium and glutathione peroxidase before and after amalgam removal in man. Acta Odontol Scand 1990;48: 189–202.
24. EPA. Water quality criterion for the protection of human health: methyl mercury. Office of Science and Technology, Office of Water, US Environmental Protection Agency, Washington DC 20460, EPA0823-R-01–001, 2001.
25. WHO. Environmental Health Criteria 1: Mercury. Geneva: World Health Organization; 1976.
26. Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, S, Dahir H. Methyl mercury poisoning in Iraq. Science 1973;181:230–241
27. Greenwood MR. Methylmercury poisoning in Iraq: an epidemiological study of the 1971–72 outbreak. J Appl Toxicol 1985;5:148–159.
28. Jernelov A. Conversion of mercury compounds. In: Miller MW, Berg GG, editors. Chemical fallout. Springfield, IL: Charles C. Thomas; 1969. p 68–74.
29. Johnels AG, Westermark T. Mercury contamination in the environment in Sweden. In: Miller MM, Berg GG, editors. Chemical Fallout. Springfield, IL: Charles C. Thomas; 1969. p 221–241.
30. McAlpine D, Araki S. Minamata disease: An unusual neurological disaster caused by contaminated fish. Lancet 1958;2:629–631
31. Irikayama, K. Case history of Minamata. In: Tsubaki T, Irukayama J, editors. Minamata disease, Tokyo: Kodansha Ltd and Elsevier Publishing Company; 1997. p 12.
32. Takeuchi T, Komyo E. The pathology of Minamata disease. Kyushu: Kyushu University Press; 1999. p 18.
33. Pfeiffer WC, Lacerda LD. Mercury inputs in the Amazon region. Environ Technol Lett 1988;9:325–330.
34. Fitzgerald WF, Clarkson TW. Mercury and monomethyl mercury: present and future concerns. Environ Health Perspect 1991;96:159–166.
35. Fitzgerald WF, Mason RP. Biogeochemical cycle of mercury in the marine environment. In: Sigel A, Sigel H, editors. Metal Ions in Biological Systems. New York: Marcel Dekker; 1997; pp 53–111.
36. Muckle G, Ayotte P, Dewailly EE, Jacobson SW, Jacobson JL. Prenatal exposure of the northern Quebec Inuit infants to environmental contaminants. Environ Health Perspect 2001 Dec;109(12):1291
37. Cernichiari E, Brewer R, Myers GJ, Marsh DO, Lapham LW, Cox C, Shamlaye CF, Berlin M, Davidson PW, Clarkson TW. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology 1995;16:705–710.
38. Amin-Zaki L, Elhassani S, Majeed MA, Clarkson TW, Doherty RA, Greenwood M. Intrauterine methyl mercury poisoning in Iraq. Pediatrics 1974;54(5):587–595.
39. Ballatori N, Clarkson TW. Biliary secretion of glutathione and glutathione-metal complexes. Fund Appl Toxicol 1985;5:816–831.
40. Kerper LE, Ballatori N, Clarkson TW. Methylmercury transport across the blood-brain barrier by an amino acid carrier. Am J Physiol 1992;267:R761–765.

41. Dutczak WJ, Ballatori N. Y-glutaml transferase dependent biliary hepatic recycling of methyl mercury in the guinea pig. J Pharmacol Exp Ther 1992;262:619–623.
42. Dutczak WJ, Ballatori N. Transport of the glutathione-methyl mercury complex across liver canalicular membranes on reduced glutathione carriers. J Biol Chem 1994;269:9746–9751.
43. Smith JC, Allen PV, Turner MD, Most B, Fisher HL, Hall LL. The kinetics of intravenously administered methyl mercury in man. Toxicol Appl Pharmacol 1994;128:251–256.
44. Magos L. Three cases of methylmercury intoxication which correct diagnosis. Arch Toxicol 1998;72:701–705.
45. Nierenberg DW, Nordgren RE, Chang MB, Siegler W, Blayney MG, Hochberg F, Toribara TY, Cernichiari E, Clarkson T. Delayed cerebellar disease and death after accidental exposure to dimethyl mercury. N Engl J Med 1998;338:1672–1675.
46. Syversen TLM. Changes in protein and RNA synthesis in rat brain after a single dose of methyl mercury. Toxicol Lett 1982;10:31–34.
47. Jacobs JM, Carmicheal N, Cavavagh JB. Ultrasructural changes in the nervous system of rabbits poisoned with methyl mercury. Toxicol Appl Pharmacol 1977;39:249–261.
48. Sarafian TA, Bredesen DE, Verity MA. Cellular resistance to methyl mercury. Neurotox 1996;17:27–36.
49. Miura K, Clarkson TW. Reduced methylmecury accumulation in a methylmercury – resistant rat pheochromocytoma PC12 cell line. Toxicol Appl Pharmacol 1993;118:39–45.
50. Davis LE, Kornfeld M, Mooney HS, Fiedler KJ, Haaland KY, Orrison WW, Cernchiari E, Clarkson TW. Methyl mercury poisoning: long term clinical, radiological, toxicological and pathological studies. Ann Neurol 1994;35:680–688.
51. Charleston JS, Body RL, Bolender RP, Mottet NK, Vahter ME, Burbacher TM. Changes in the number of astrocytes and microglia in the thalamus of the monkey *Macaca fascicularis* following long-term subclinical methylmercury exposure. Neurotoxicology 1996;17:127–138.
52. Magos L, Brown AW, Sparrow S, Bailey E, Snowden RE, Skipp WR. The comparative toxicology of ethyl- and methyl mercury. Arch Toxicol 1985;57:260–267.
53. Takeuchi T. Pathology of Minamata disease: the effect of methyl mercury on interuterine life of human beings. Pediatrician 1977;6:69–87.
54. Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Al-Tikriti S. Fetal methylmercury poisoning: relationship between concentration in single strands of maternal hair and child effects. Arch Neurol 1987;44:1017–1022.
55. Cox C, Clarkson TW, Marsh DO, Amin-Zaki L, Al-Tikriti S, Myers G. Dose-response analysis of infants prenatally exposed to methyl mercury: an application of a single compartment model to single-strand hair analysis. Environ Res 1989;49:318–332.
56. Swedish Expert Group. Methylmercury in fish. A toxicological-epidemiological evaluation of risks. Nord Hyg Tidskr 1971;4(suppl):19–364.
57. Watanabe C, Satoh H. Evolution of our understanding of methylmercury as a health threat. Environ Health Perspect 1996;104:367–379.
58. Moriyama H, Takizawa. Assessing low-dose effects on children exposed in utero to methylmercury—Protocol for on-the-spot re-inspection for Minamata and Niigata data at early stage. In: Takizawa Y, Oame M, editors. Understanding of Minamata disease: methlymercury poisoning in Minamata and Niigata, Japan. Tokyo: Japan Public Health Association.
59. McKeown-Eyssen GE, Ruedy J, Neims A. Methylmercury exposure in northern Quebec. II. Neurological findings in children. Am J Epidemiol 1983;118:470–479.
60. Kjellstrom T, Kennedy P, Wallis S, Mantell C. Phyical and mental development of children with prenatal exposure to mercury from fish. Stage 1. Preliminary tests at age 4. Solna: National Swedish Environmental Board 96 pp (Report No. 3080); 1986.
61. Kjellstrom T, Kennedy P, Wallis S, Stewart A, Friberg L, Lind B, Wutherspoon P, Mantell C. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2. Interviews and psychological tests at age 6. Solna: National Swedish Environmental Board (Report No. 3642); 1989. 112 p.
62. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. Cognitive deficit in 7 year old children with prenatal exposure to methyl mercury. Neurotoxicol Teratol 1997;6:417–428.

63. Davidson PW, Kost J, Myers GJ, Cox C, Clarkson TW, Shamlaye CF. and neurodevelopment: reanalysis of the Seychelles Child Development Study outcomes at 66 months of age. JAMA 2001;285:1291–1293.
64. Choi BH, Lapham LW, Amin-Zaki L, Saleem T. Abnormal neuronal migration, deranged cerebellar cortical organization, and diffuse white matter astrocytosis of human fetal brain. A major effect of methyl mercury poisoning in utero. J Neuropathol Neurol 1978;37:719–733.
65. Miura K, Imura N. Mechanism of methyl mercury cyto-toxicity. Crit Rev Toxicol 1987; 18:161–188.
66. Rodier PM, Ashner M, Sager PR. Mitotic arrest in the developing CNS after prenatal exposure to methyl mercury. Neurobehav Toxicol Teratol 1984;6:379–385.
67. Philbert MA, Billingsley ML, Reuhl KR. Mechanisms of injury in the central nervous system. Toxicol Pathol 2000;28:43–53.
68. AAP and USPHS. Joint statement of the American Academy of Pediatrics (AAP) and the United States Public Health Service (USPHS). Pediatrics 1999;104:568–569.
69. Ball LK, Ball R, Pratt RD. An assessment of Thimerosal use in childhood vaccines. Pediatrics 2001;107:1147–1154.
70. Pless R, Risher JF. Mercury, infant neurodevelopment, and vaccination. J Pediatr 2000; 136:571–573.
71. Seal JB, Daum RS. What happened to Primum Non Nocere. Pediatrics 2001;107:1177–1178.
72. Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a Novel form of Mercury Poisoning. Cranford, NJ: ARAC Research; 2000. p 1–11.
73. Al-Damluji S. Mercurial poisoning with the fungicide granosan M. J Faculty Med (Baghdad) 1962;4:82–103.
74. Jalili MA, Abbasi AH. Poisoning by ethyl mercury toluene sulfananilade. Br J Ind Med 1961; 18:303–308.
75. Zhang J. Clinical observations in ethyl mercury chloride poisoning. Am J Ind Med 1984; 5:251–258.
76. Axton JHM. Six cases of poisoning after a parenteral organic mercurial compound (Merthiolate). Postgrad Med J 1972;48:417–421.
77. Rohyans J, Walson PD, Wood GA, MacDonald WA. Mercury toxicity following merthiolate ear irrigations. Pediatrics 1984;104:311–313.
78. Pfab R, Muckter H, Roider G, Zilker T. Clinical course of severe poisoning with Thimerosal. Clin Toxicol 1996;34:453–460.
79. Fagan DG, Pritchard JS, Clarkson TW, Greenwood MR. Organ mercury levels in infants with omphaloceles treated with organic mercurial antiseptic. Arch Dis Child 1977;52:962–964.
80. Matheson DS, Clarkson TW, Gelfand EW. Mercury toxicity (acrodynia) induced by long-term injection of gamma globulin. J Pediatr 1980;97:153–155.
81. Zemtsov A, Bolton GG. Thimerosal-induced bullous contact dermatitis. Contact Dermatitis 1994;30:57.
82. Zenorala P, Gimma A, Lomuto M. Systemic contact dermatitis from Thimerosal. Contact Dermatitis 1995;32:107–108.
83. Cox NH, Forsyth A. Thimerosal and vaccination reactions. Contact Dermatitis 1988;18: 229–233.
84. McKenna KE. Eczematous reaction to hepatitis B vaccine. Contact Dermatitis 1999;40: 158–159.
85. EPA. Mercury report to Congress, Office of Air Quality and Standards, Washington, DC: US Environmental Protection Agency; 1997.
86. U.S. EPA Methylmercury (MeHg) Iris Summary. 2002. http://www.epa.gov/iris/subst/0073.htm
87. Stajich GV, Lopez GP, Harry SW, Sexson WR. Iatrogenic exposure to mercury after hepatitis B vaccination in pre-term infants. J Pediatr 2000;136:679–681.
88. Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: a descriptive study. Lancet 2002;360: 1737–1741.
89. Magos L. Review on the Toxicity of ethyl mercury including its presence as a preservative in biological and pharmaceutical preparations. J Appl Toxicol 2001;21:1–5.

90. Wantke F, Demmer CM, Gotz M, Jarisch RR. Contact dermatitis from thimerosal. 2 years experience with ethylmercuric chloride in patach testing thimerosal-sensitive patients. Contact Dermatitis 1994;30:115–117.
91. Santucci B, Cannistraci C, Cristaudo A, Camera E, Picardo M. Thimerosal positivities: the role of organomercury alkyl compounds. Contact Dermatitis 1998;38:325–328.
92. Goncalo M, Figueiredo A, Goncalo S. Contact Dermatitis 1996;34:201–203.
93. WHO. Recommendations from the Strategic Advisory Group of Experts. Weekly Epidemiol Rec 2002;77:305–316.

3

REVIEW ARTICLE

CURRENT CONCEPTS

# The Toxicology of Mercury — Current Exposures and Clinical Manifestations

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MERCURY HAS BEEN USED COMMERCIALLY AND MEDICALLY FOR CENturies. In the past it was a common constituent of many medications. It is still used in hospitals in thermometers and blood-pressure cuffs and commercially in batteries, switches, and fluorescent light bulbs. Large quantities of metallic mercury are employed as electrodes in the electrolytic production of chlorine and sodium hydroxide from saline. These uses still give rise to accidental and occupational exposures.[1]

Today, however, exposure of the general population comes from three major sources: fish consumption, dental amalgams, and vaccines. Each has its own characteristic form of mercury and distinctive toxicologic profile and clinical symptoms. Dental amalgams emit mercury vapor that is inhaled and absorbed into the bloodstream. Dentists and anyone with an amalgam filling are exposed to this form of mercury. Liquid metallic mercury (quicksilver) still finds its way into homes, causing a risk of poisoning from the vapor and creating major cleanup costs. Humans are also exposed to two distinct but related organic forms, methyl mercury ($CH_3Hg^+$) and ethyl mercury ($CH_3CH_2Hg^+$). Fish are the main if not the only source of methyl mercury, since it is no longer used as a fungicide. In many countries, babies are exposed to ethyl mercury through vaccination, since this form is the active ingredient of the preservative thimerosal used in vaccines. Whereas removal of certain forms of mercury, such as that in blood-pressure cuffs, will not cause increased health risks, removal of each of the three major sources described in this article entails health risks and thus poses a dilemma to the health professional.

Exposure to mercury from dental amalgams and fish consumption has been a concern for decades, but the possible risk associated with thimerosal is a much newer concern. These fears have been heightened by a recent recommendation by the Environmental Protection Agency (EPA) that the allowable or safe daily intake of methyl mercury be reduced from 0.5 μg of mercury per kilogram of body weight per day, the threshold established by the World Health Organization in 1978,[2] to 0.1 μg of mercury per kilogram per day.[3]

Table 1 summarizes the clinical toxicologic features of mercury vapor and methyl and ethyl mercury. It also includes data on inorganic divalent mercury, since this is believed to be the toxic species produced in tissues after inhalation of the vapor.[5] It is also responsible for kidney damage after exposure to ethyl mercury, since ethyl mercury is rapidly converted to the inorganic form.[13] Inorganic mercury as both mercuric and mercurous salts was also the chief cause of acrodynia, a childhood disease that is now mainly of historical interest.[14] The clinical symptoms of acrodynia consist of painful, red, swollen fingers and toes in association with photophobia, irritability, asthenia, and hypertension. It is believed to be a hypersensitivity reaction.

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Table 1. The Major Clinical Toxicologic Features of Mercury.*

| Variable | Mercury Vapor | Inorganic Divalent Mercury | Methyl Mercury | Ethyl Mercury |
|---|---|---|---|---|
| Route of exposure | Inhalation | Oral | Oral (from fish consumption) | Parenteral (through vaccines) |
| Target organ | Central nervous system, peripheral nervous system, kidney | Kidney | Central nervous system | Central nervous system, kidney |
| Local clinical signs | | | | |
| Lungs | Bronchial irritation, pneumonitis (>1000 $\mu g/m^3$ of air) | | | |
| Gastrointestinal tract | Metallic taste, stomatitis, gingivitis, increased salivation (>1000 $\mu g/m^3$ of air) | Metallic taste, stomatitis, gastroenteritis | | |
| Skin | | Urticaria, vesication | | |
| Systemic clinical signs | | | | |
| Kidney | Proteinuria (>500 $\mu g/m^3$ of air) | Proteinuria, tubular necrosis | | Tubular necrosis |
| Peripheral nervous system | Peripheral neuropathy (>500 $\mu g/m^3$ of air) | Acrodynia | | Acrodynia |
| Central nervous system | Erethism (>500 $\mu g/m^3$ of air), tremor | | Paresthesia, ataxia, visual and hearing loss (>200 $\mu$g/liter of blood) | Paresthesia, ataxia, visual and hearing loss |
| Approximate half-life (whole body) (days) | 60 | 40 | 70 | 20† |
| Treatment‡ | Meso-2,3-dimercaptosuccinic acid | Meso-2,3-dimercaptosuccinic acid | Chelators not effective§ | Chelators not effective§ |

* Data were adapted from Gossel and Bricker.[4] Clinical manifestations vary with the degree and length of exposure. The values in parentheses are the approximate range of mercury concentration in air (expressed as micrograms per cubic meter) and in blood (expressed as micrograms per liter) associated with the onset of clinical signs and symptoms. Epidemiologic studies that did not use specific end points such as IQ score indicate a risk of adverse effects (approximately 5 percent) at lower concentrations (e.g., 25 to 50 $\mu$g of mercury vapor per cubic meter and 40 $\mu$g of methyl mercury per liter of blood are associated with an increased risk of prenatal damage to the developing central nervous system).[3,5] In general, the atmospheric concentration of mercury vapor equals the urinary concentration. The mean urinary concentration in the U.S. general population is 0.72 $\mu$g per liter (95 percent confidence interval, 0.6 to 0.8), and the mean blood concentration is 0.34 $\mu$g per liter (95 percent confidence interval, 0.3 to 0.4).[6] In Europe[7] and other parts of the world,[8] blood concentrations appear to be somewhat higher. The mean urinary concentrations increase according to the number of dental amalgam surfaces, and blood concentrations increase according to the level of fish consumption.[6] No reliable data are available on the concentration of inorganic divalent mercury associated with adverse effects.

† The half-life in blood is about 20 days in adults but may be as short as 7 days in infants.

‡ Details of meso-2,3-dimercaptosuccinic acid treatment have been published.[9-11]

§ Chelators can remove methyl and ethyl mercury from the body; they cannot reverse the damage to the central nervous system. They may, however, prevent further deterioration.[12]

## MERCURY VAPOR FROM DENTAL AMALGAMS

Dental amalgams have been in use for over 150 years. They are inexpensive and thought to be more durable and easier to use than other types of fillings. The amalgam consists of approximately 50 percent mercury combined with other metals such as silver and copper. Since their introduction, dental amalgams have been a source of controversy because of the assumed health risks of mercury. The arguments between the protagonists and antagonists have been referred to as the "amalgam wars" and became more heated around 1970 with the discovery that amalgams can release mercury vapor into the oral cavity in concentrations that are higher than those deemed safe by occupational health guidelines.

Subsequently, it was realized that the actual inhaled dose was small, owing to the small volume of the oral cavity. Nevertheless, amalgam fillings are the chief source of exposure to mercury vapor in the general population.[8] Brain, blood, and urinary concentrations correlate with the number of amalgam surfaces present. It has been estimated that 10 amalgam surfaces would raise urinary concentrations by 1 µg of mercury per liter, roughly doubling the background concentrations.[15] Higher urinary concentrations are found in persons who chew a great deal. For example, the long-term use of nicotine chewing gum will raise urinary concentrations close to occupational health limits.[16] The removal of amalgam fillings can also cause temporary elevations in blood concentrations,[17] since the process transiently increases the amount of mercury vapor inhaled.

What is the health risk from such exposures? Cases of poisoning from inhalation of mercury vapor have been recognized for centuries.[18] Severe cases are characterized by a triad of intentional tremor, gingivitis, and erethism (Table 1). Erethism consists of bizarre behavior such as excessive shyness and even aggression. The Mad Hatter in *Alice in Wonderland* was probably a victim of occupational mercury intoxication.

Today's occupational exposures, such as in the dental office, are lower and may lead to mild, reversible effects on the kidney or mild cognitive changes and memory loss.[5] However, urinary concentrations in people with amalgams (about 2 to 4 μg of mercury per liter) are well below concentrations found in people who are occupationally exposed to mercury (20 to 50 μg of mercury per liter) unless they are also excessive chewers. Current concern arises from claims that long-term exposure to low concentrations of mercury vapor from amalgams either causes or exacerbates degenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis, and Parkinson's disease. Speculation has been most intense with respect to Alzheimer's disease after a report that the brains of patients with Alzheimer's disease had elevated mercury concentrations. However, several epidemiologic investigations failed to provide evidence of a role of amalgam in these degenerative diseases, including a long-term study of 1462 women in Sweden,[19] an ongoing Swedish twin study involving 587 subjects,[20] and a study of 129 nuns 75 to 102 years of age, which included eight tests of cognitive function.[21] Nevertheless, in vitro studies have indicated that mercury can affect the biochemical processes believed to be involved in Alzheimer's disease.[22] The problem is that mercury can inhibit various biochemical processes in vitro without having the same effect in vivo.

Patients who have questions about the potential relation between mercury and degenerative diseases can be assured that the available evidence shows no connection. Some will ask whether their mercury fillings should be removed. They should be reminded that the process of removal generates mercury vapor and that blood concentrations will subsequently rise substantially before they eventually decline.[17] There is no clear evidence supporting the removal of amalgams.

## MERCURY VAPOR FROM QUICKSILVER IN THE HOME

Recent attempts by power companies to replace pressure-control devices for the domestic gas supply have led to spills of liquid mercury, affecting some 200,000 homes in one incident.[23] Spills of liquid mercury in the home carry a risk of vapor inhalation. Quicksilver is an attractive play object for children and is found in many homes, especially in developing countries. High levels of exposure to mercury vapor can result from the cultural and religious use of elemental mercury, including sprinkling mercury on the floor of a home or car, burning it in a candle, and mixing it with perfume.[24]

Infants and young children, whose breathing zones are closest to the floor, are at highest risk, since mercury vapor is heavy and tends to form layers close to the floor. Ingested liquid mercury passes through the gastrointestinal tract essentially unabsorbed. Centuries ago a tablespoonful of quicksilver was used to treat constipation.[25] It arguably represents one of the first uses of gravity in medicine.

## METHYL MERCURY

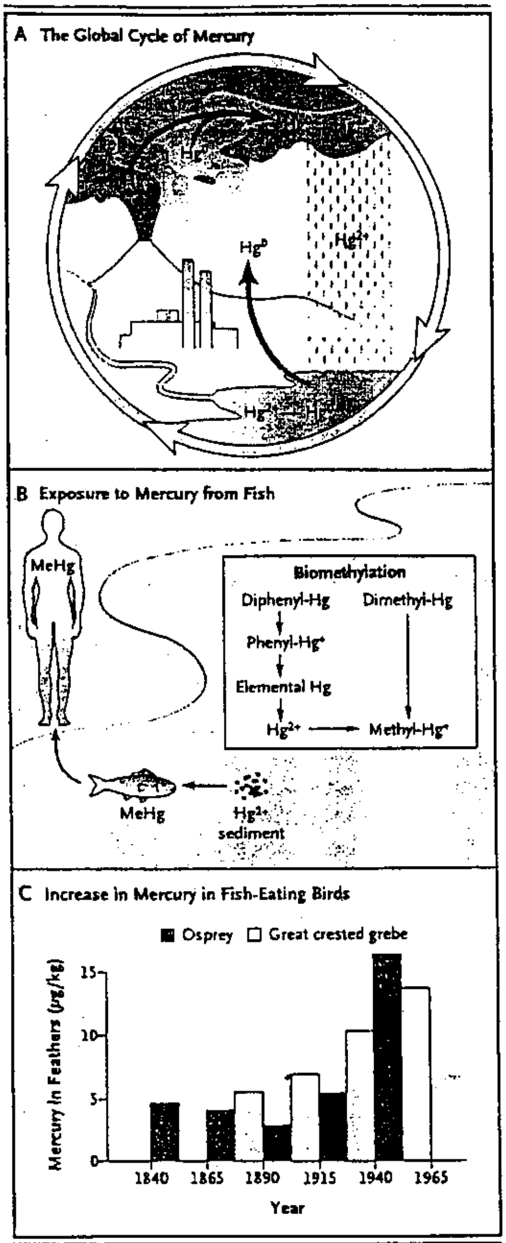
Among humans, the sole source of exposure to methyl mercury is the consumption of fish and sea mammals. Methyl mercury is produced environmentally by biomethylation of the inorganic mercury present in aquatic sediments (Fig. 1). It accumulates in the aquatic food chain and reaches its highest concentrations in long-lived, predatory fish such as swordfish and shark in the oceans and pike and bass in fresh water. Concentrations of mercury in ambient air and water are too low to pose a serious risk to the general population.

### EXPOSURE IN ADULTS

Cases of severe, even fatal, methyl mercury poisoning date back to the 1860s in England, when such mercurials were first synthesized.[28] Subsequent cases arose through occupational and dietary exposures. Several large outbreaks were caused by the consumption of bread mistakenly made from methyl mercury–coated seed grain; for example, an out-

**Figure 1. The Global Cycle of Mercury.**

In nature, mercury vapor ($Hg^0$), a stable monatomic gas, evaporates from the earth's surface (both soil and water) and is emitted by volcanoes (Panel A). Anthropogenic sources include emissions from coal-burning power stations and municipal incinerators. After approximately one year, mercury vapor is converted to a soluble form ($Hg^{2+}$) and returned to the earth in rainwater. It may be converted back to the vapor form both in soil and in water by microorganisms and reemitted into the atmosphere. Thus, mercury may recirculate for long periods. Mercury attached to aquatic sediments is subject to microbial conversion to methyl mercury (MeHg), whereupon it enters the aquatic food chain. It reaches its highest concentrations in long-lived predatory fish, such as sharks. Panel B indicates the routes of transformation to methyl mercury as originally suggested by Jernelöv.[26] Panel C depicts the increase in mercury concentrations in feathers of fish-eating birds in Sweden.[27] The period covered by these data corresponds approximately to the growth of industrialization in Sweden.




break in 1971 and 1972 in Iraq caused hundreds of deaths and thousands of cases of severe intoxication.[29] The industrial release of methyl mercury into Minamata Bay and the Agano River in Japan resulted in the accumulation of the toxicant in fish and, subsequently, in two large epidemics related to fish consumption.[30] Overt cases of poisoning are now rare. In the United States, the only reported cases in the past 35 years involved a family that consumed the meat of a pig fed treated grain[31] and a university professor who was accidentally exposed in the laboratory.[11]

The brain is the primary target tissue. Adults present with paresthesias of the circumoral area and hands and feet, followed by visual-field constriction and ataxia. Neuropathological examination reveals regional destruction of neurons in the visual cortex and cerebellar granule cells. There is usually a latent period of weeks or months between exposure and the onset of symptoms.

Several studies have reported statistical associations between cardiovascular disease and mercury, mostly in the form of methyl mercury. One study found a direct relation between mercury concentrations and the risk of myocardial infarction,[32] whereas a nested case–control study of more than 300,000 health professionals found no such association.[33] A third study, from eastern Finland, where the consumption of saturated animal fat is high, found an association, but the authors suggested that their finding might be specific to the region.[34,35] A fourth study among seven-year-old children on the Faeroe Islands found that blood pressure was increased when the blood mercury concentration was below 10 µg per liter but not when it was higher.[36] "Contrary to expectation," as the authors stated, "this association occurred within an exposure range characteristic of communities not depending on marine food" such as the United States.[37] They also pointed out that "the average birth weight in this fishing community is the highest in the world and therefore the community may represent a unique setting."

Thus, firm conclusions about cause and effect cannot be yet made, since cardiovascular disease has multiple risk factors (e.g., family history, stress, dietary habits, smoking, alcohol use, diabetes, and socioeconomic status). The researchers themselves recognize this complication and use extensive statistical measures to correct for these factors. Prospective studies are needed to settle this issue.[38]

#### PRENATAL EXPOSURE

The fetal brain is more susceptible than the adult brain to mercury-induced damage. Methyl mercury inhibits the division and migration of neuronal cells and disrupts the cytoarchitecture of the developing brain. In the past 15 years or so, epidemiologic studies have focused on the effects of prenatal exposure.[39-41] As a consequence of these epidemiologic data, the EPA reduced the allowable intake of methyl mercury from 0.5 to 0.1 μg of mercury per kilogram per day.[42] This threshold is lower than those used by other regulatory agencies. Moreover, it translates into a weekly consumption of one 198-g (7-oz) can of tuna for an adult. Given that canned tuna is the cheapest and most widely consumed fish in the United States and is approved by the American Heart Association as part of a diet low in saturated fat and cholesterol, the debate over the safety of tuna and fish in general will continue with some intensity.

It is reassuring that the only clinical reports of mercury poisoning from fish consumption are those from Japan in the 1950s and 1960s.[8] The EPA guideline is derived from reports of subtle and small neuropsychological changes in children in the Faeroe Islands study, whose exposure was mainly from whale consumption.[36] A similar study in the Seychelles found no adverse effects from fish consumption alone.[41] The majority of the general population in the United States has levels of exposure well below the EPA guideline, but 8 percent or so have levels that are slightly higher. Although a National Academy of Sciences committee reported that 60,000 children in the United States were at risk as a result of prenatal exposure,[43] they failed to provide any justification or explanation for that conclusion.

Fish consumption has clear health benefits, and the risk posed by exposure to mercury is currently speculative. The Food and Drug Administration has recommended that pregnant women, nursing mothers, and young children avoid eating fish with a high mercury content (>1 ppm), such as shark, swordfish, tilefish, and king mackerel. Because whale meat contains up to 3 ppm of mercury, about half of which is in the form of methyl mercury,[44] consumption of whale meat should also be discouraged.

### THIMEROSAL IN VACCINES

Thimerosal has been used as a preservative in many vaccines since the 1930s.[45,46] At concentrations found in vaccines, thimerosal meets the requirements for a preservative set forth by the U.S. Pharmacopeia[47] — that is, it kills the specified challenge organisms and can prevent the growth of the challenge fungi. It contains the ethyl mercury radical ($CH_3CH_2Hg^+$) attached to the sulfur group of thiosalicylate and is believed to behave toxicologically like other ethyl mercury compounds. Early toxicity studies found no adverse health effects; recently, however, Ball et al. reevaluated thimerosal by applying the revised EPA guideline for methyl mercury to ethyl mercury.[48] They calculated that infants undergoing the usual U.S. program of vaccines from birth to six months of age would receive more than 0.1 μg of mercury per kilogram per day.[8] Steps were rapidly taken to remove thimerosal from vaccines by switching to single-dose vials that did not require any preservative. This process is now virtually complete in the United States. The decision itself is remarkable, and the speed of execution even more so[49]; however, the EPA guideline is based on epidemiologic data on prenatal exposure to methyl mercury rather than postnatal exposure to ethyl mercury. Ethyl mercury has some similarities to methyl mercury. They are closely related chemically, have a similar initial distribution in the body, and cause similar types of damage to the brain in toxic doses.

They also have differences. Methyl mercury is more potent. Ethyl mercury is metabolized more rapidly to inorganic mercury; perhaps this is why ethyl mercury, unlike methyl mercury, causes kidney damage in humans. Of greater importance is the recent finding that the half-life of ethyl mercury in the body is much shorter.[50] The half-life of methyl mercury in blood, which is assumed to indicate the total body burden, is usually assumed to be about 50 days.[51] In contrast, in children receiving thimerosal in vaccines, the half-life of ethyl mercury in blood was 7 to 10 days, or 1/7 to 1/5 as long as that of methyl mercury.[50] Therefore, in the two-month periods between vaccinations (at birth and at two, four, and six months), all of the mercury should have been excreted, so that there is no accumulation.

Given the short half-life of ethyl mercury, any risks of its damaging either the brain or kidneys would seem remote. A World Health Organization advisory committee recently concluded that it is safe to continue using thimerosal in vaccines.[52] This is especially important in developing countries, where the use of a preservative is essential in multidose vials. The known risk of infectious diseases far exceeds that of the hypothetical risk of thimerosal. Claims have been made that thimerosal in vaccines may be a cause of autism and related disorders, but studies testing that theory have yet to be performed.

All forms of mercury have adverse effects on health at high doses. However, the evidence that exposure to very low doses of mercury from fish consumption, the receipt of dental amalgams, or thimerosal in vaccines has adverse effects is open to wide interpretation. Moreover, attempts to reduce such exposure may pose greater health risks than those hypothesized to occur from mercury.

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REFERENCES

1. Toxicological profile for mercury. Atlanta: Agency for Toxic Substances Disease Registry, 1999.
2. Evaluation of certain food additives and contaminants: twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organ Tech Rep Ser 1978;631:1-39.
3. Environmental Protection Agency. Reference dose for chronic oral exposure to methylmercury. Greenbelt, Md.: Integrated Risk Information System, 2001.
4. Gossel TA, Bricker JD. Principles of clinical toxicology. 2nd ed. New York: Raven Press, 1990.
5. Inorganic mercury. Vol. 118 of Environmental health criteria. Geneva: World Health Organization, 1991.
6. Second national report on human exposure to environmental chemicals. Atlanta: Centers for Disease Control and Prevention, 2003. (Accessed October 6, 2003, at http://www.cdc.gov/exposurereport/.)
7. Brune D, Nordberg GF, Vesterberg O, Gerhardsson L, Wester PO. A review of normal concentrations of mercury in human blood. Sci Total Environ 1991;100:235-82.
8. Methylmercury. Vol. 101 of Environmental health criteria. Geneva: World Health Organization, 1990.
9. Forman J, Moline J, Cernichiari E, et al. A cluster of pediatric metallic mercury exposure cases treated with meso-2,3-dimercaptosuccinic acid (DMSA). Environ Health Perspect 2000;108:575-7.
10. Bluhm RE, Bobbitt RG, Welch LW, et al. Elemental mercury vapour toxicity, treatment, and prognosis after acute, intensive exposure in chloralkali plant workers. I. History, neuropsychological findings and chelator effects. Hum Exp Toxicol 1992;11:201-10.
11. Nierenberg DW, Nordgren RE, Chang MB, et al. Delayed cerebellar disease and death after accidental exposure to dimethylmercury. N Engl J Med 1998;338:1672-6.
12. Pfab R, Muckter H, Roider G, Zilker T. Clinical course of severe poisoning with thiomersal. J Toxicol Clin Toxicol 1996;34:453-60.
13. Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. The comparative toxicology of ethyl- and methylmercury. Arch Toxicol 1985;57:260-7.
14. Warkany J, Hubbard DM. Acrodynia and mercury. J Pediatr 1953;42:365-86.
15. Kingman A, Albertini T, Brown LJ. Mercury concentrations in urine and whole blood associated with amalgam exposure in a US military population. J Dent Res 1998;77:461-71.
16. Sallsten G, Thoren J, Barregard L, Schutz A, Skarping G. Long-term use of nicotine chewing gum and mercury exposure from dental amalgam fillings. J Dent Res 1996;75:594-8.
17. Molin M, Bergman B, Marklund SL, Schutz A, Skerfving S. Mercury, selenium, and glutathione peroxidase before and after amalgam removal in man. Acta Odontol Scand 1990;48:189-202.
18. Ramazzini B. Diseases of workers. Wright WC, trans. New York: Hafner Publishing, 1964.
19. Ahlqwist M, Bengtsson C, Lapidus L, Gergdahl IA, Schutz A. Serum mercury concentration in relation to survival, symptoms, and disease: results from the prospective population study of women in Gothenburg, Sweden. Acta Odontol Scand 1999;57:168-74.
20. Bjorkman L, Pedersen NL, Lichtenstein P. Physical and mental health related to dental amalgam fillings in Swedish twins. Community Dent Oral Epidemiol 1996;24:260-7.
21. Saxe SR, Snowdon DA, Wekstein MW, et al. Dental amalgam and cognitive function in older women: findings from the Nun Study. J Am Dent Assoc 1995;126:1495-501.
22. Leong CC, Syed NI, Lorscheider FL. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury. Neuroreport 2001;12:733-7.
23. Gibson R, Taylor TS. Nicor says mercury spilled at more sites: contamination found at 6 new locations, company tells state. Chicago Tribune (Sports Final Edition Section, zone N). September 14, 2000:1.
24. Riley DM, Newby CA, Leal-Almeraz TO, Thomas VM. Assessing elemental mercury vapor exposure from cultural and religious practices. Environ Health Perspect 2001;109:779-84.
25. Goldwater LJ. Mercury: a history of quicksilver. Baltimore: York Press, 1972.
26. Jernelöv A. Conversion of mercury compounds. In: Miller MW, Berg GG, eds. Chemical fallout: current research on persistent pesticides. Springfield, Ill.: Charles C Thomas, 1969:68-74.
27. Johnels AG, Westermark T. Mercury contamination of the environment in Sweden. In: Miller MW, Berg GG, eds. Chemical fallout: current research on persistent pesticides. Springfield, Ill.: Charles C Thomas, 1969:221-41.
28. Hunter D. The diseases of occupations. 4th ed. London: English Universities Press, 1969:314-28.
29. Bakir F, Damluji SF, Amin-Zaki L, et al. Methylmercury poisoning in Iraq. Science 1973;181:230-41.
30. Eto K, Oyanagi S, Itai Y, Tokunaga H, Takizawa Y, Suda I. A fetal type of Minamata disease: an autopsy case report with special reference to the nervous system. Mol Chem Neuropathol 1992;16:171-86.
31. Likosky WH, Hinman AR, Barthel WF. Organic mercury poisoning, New Mexico. Neurology 1970;20:401.
32. Guallar E, Sanz-Gallardo MI, van't Veer P, et al. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 2002;347:1747-54.
33. Yoshizawa K, Rimm EB, Morris JS, et al. Mercury and the risk of coronary heart disease in men. N Engl J Med 2002;347:1755-60.
34. Salonen JT, Seppanen K, Lakka TA, Salonen R, Kaplan GA. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population-based prospective 4-year follow-up study in men in eastern Finland. Atherosclerosis 2000;148:265-73.
35. Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarc-

tion and coronary, cardiovascular, and any death in eastern Finnish men. Circulation 1995;91:645-55.

36. Sorensen N, Murata K, Budtz-Jorgensen E, Weihe P, Grandjean P. Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. Epidemiology 1999;10:370-5.

37. Blood and hair mercury levels in young children and women of childbearing age — United States, 1999. JAMA 2001;285:1436-7.

38. Bolger PM, Schwetz BA. Mercury and health. N Engl J Med 2002;347:1735-6.

39. Kjellstrom T, Kennedy P, Wallis S, et al. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2. Interviews and psychological tests at age 6. Report no. 3642. Solna, Sweden: Solna National Swedish Environmental Board, 1989.

40. Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 1997;19:417-28.

41. Myers GJ, Davidson PW, Cox C, et al. Prenatal methylmercury exposure from ocean fish consumption: 9-year evaluations in the Seychelles child development study. Lancet 2003;361:1686-92.

42. Water quality criterion for the protection of human health: methylmercury. Washington, D.C.: Environmental Protection Agency, January 2001. (EPA-823-R-01-001.)

43. Board on Environment studies and toxicology. Toxicological effects of methyl mercury. Washington, D.C.: National Research Council, 2000.

44. Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, Videro T. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch Environ Health 1991;47:185-95.

45. Magos L. Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical preparations. J Appl Toxicol 2001;21:1-5.

46. Clarkson TW. The three modern faces of mercury. Environ Health Perspect 2002;110: Suppl 1:11-23.

47. USP 24–NF 19: U.S. Pharmacopeia and national formulary. 24th ed. Rockville, Md.: United States Pharmacopeial Convention, 1999.

48. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. Pediatrics 2001;107:1147-54.

49. Freed GL, Andreae MC, Cowan AE, Katz SL. The process of public policy formulation: the case of thimerosal in vaccine. Pediatrics 2002;109:1153-9.

50. Pichichero MB, Cernichiari E, Lopricato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. Lancet 2002;360:1737-41.

51. Smith J, Farris FF. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol 1996;137:245-52.

52. Vaccines and biologicals: recommendations from the Strategic Advisory Group of Experts. Wkly Epidemiol Rec 2002;77:305-12.

4

# Thimerosal in Childhood Vaccines, Neurodevelopment Disorders, and Heart Disease in the United States

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**Abstract**

In this study, we evaluated doses of mercury from thimerosal-containing childhood immunizations in comparison to US Federal Safety Guidelines and the effects of increasing doses of mercury on the incidence of neurodevelopment disorders and heart disease. This study showed that children received mercury from this source in excess of the Federal Safety Guidelines for the oral ingestion of methylmercury. Our analyses showed increasing relative risks for neurodevelopment disorders and heart disease with increasing doses of mercury. This study provides strong epidemiological evidence for a link between mercury exposure from thimerosal-containing childhood vaccines and neurodevelopment disorders.

**Introduction**

Many sources now confirm an autism epidemic in the United States. The prevalence of autism has risen from one in about 2,500 children in the mid-1980s to one in about 300 children in 1996.[1,2,3] Several studies report that there is an association between mercury exposure and an increased risk of heart disease.[4,5] Many in the scientific/medical community have, initially, been highly skeptical that thimerosal, an ethylmercury preservative, in childhood vaccines could be associated with neurodevelopment disorders.

Thimerosal is an organic mercury compound. It is metabolized to ethylmercury and thiosalicylate and has been present since the 1930s as a preservative in many vaccines and pharmaceutical products to prevent bacterial and fungal contamination.

In 2001, the Institute of Medicine (IOM) of the US National Academy of Sciences concluded that the hypothesis that exposure to thimerosal-containing vaccines could be associated with neurodevelopment disorders is not established and rests on indirect and incomplete information, primarily from analogies with methylmercury and levels of maximum mercury exposure from vaccines given in children. They concluded that the hypothesis is biologically possible, but the possible relationship between thimerosal from vaccines and neurodevelopment disorders of autism, attention deficit/hyperactivity disorder (ADHD), and speech or language delay remained seriously suspect.[7]

Since the publication of the IOM report, we published the first epidemiological evidence showing a direct association between thimerosal-containing childhood vaccines and neurodevelopment disorders in children.[8] We showed that there was from a 2 to 6-fold increased incidence of neurodevelopment disorders following an additional 75-100µg dosage of mercury from thimerosal-containing childhood vaccines in comparison to thimerosal-free childhood vaccines.

As the first part of this study, we evaluated the doses of mercury that children received from thimerosal-containing vaccines, as part of the routine US childhood immunization schedule, in comparison to the US Federal Safety Guidelines for the oral ingestion of methylmercury. In 1999, the US Food and Drug Administration (FDA) determined that under the recommended childhood immunization schedule infants might be exposed to cumulative doses of ethylmercury that exceed some federal safety guidelines established for the oral ingestion of methylmercury.[9]

Secondly, in order to analyze the effects of thimerosal in vaccine recipients, we analyzed the incidence rates of neurodevelopment disorders and heart disease reported following thimerosal-containing vaccines in comparison to thimerosal-free vaccines based upon analysis of the Vaccine Adverse Events Reporting System (VAERS) database. We analyzed thimerosal-containing Diphtheria-Tetanus-whole-cell-Pertussis (DTwcP) and Diphtheria-Tetanus-acellular-Pertussis (DTaP) vaccines in comparison to thimerosal-free DTaP vaccines.

Finally, we analyzed data from the US Department of Education on the number of children of various ages in US schools who were reported with various types of disabilities in comparison to the mercury dose that children received from thimerosal in their childhood vaccines.

**Methods**

EPA/FDA Exposure Limits

In this study, the amount of mercury children received as part of their routine childhood immunization schedule and the EPA and FDA maximum permissible doses for the oral ingestion of methylmercury were determined from the IOM report.[7] The maximum permissible doses for the oral ingestion of methylmercury by the EPA and FDA are 0.1 µg /kg body weight/day and 0.4 µg /kg body weight/day, respectively. The average size of infants at various ages was determined from Geigy Scientific Tables.[10]

The VAERS Database

The incidence of neurodevelopment disorders and heart disease following thimerosal-containing DTaP and DTwcP vaccines in comparison to thimerosal-free DTaP vaccines was based upon analysis of the VAERS database, using Microsoft Access.®

The VAERS database is an epidemiologic database maintained by the Centers for Disease Control and Prevention (CDC) since 1990. All adverse reactions are to be reported to the VAERS database as required by US law. The CDC requires written and telephonic confirmation of serious adverse reactions and follows up these patients one year later. The FDA inquires into deaths reported to the VAERS database by contacting the patient's healthcare provider and physician. The FDA also continually monitors reports to the VAERS database to determine whether any vaccine or vaccine lot has a higher than expected incidence rate of events. The VAERS Working Group of the CDC, the FDA, and we analyze and publish epidemiologic studies based upon analysis of the VAERS database.

The neurodevelopment disorders and heart disease conditions we analyzed were autism, speech disorders, and heart arrest. These categories of adverse events were based upon descriptions of adverse reactions by those reporting them and by defined fields contained in the VAERS database. In addition, as control adverse events we analyzed the number of febrile seizures, fevers, pain, edema, and vomiting following each of the vaccines under study. We determined the number of each type of adverse event reported following doses for two groups of patients, the first receiving an average of 37.5 µg of mercury and the second, an average of 87.5 µg of mercury. This grouping allowed us to be able to ascertain larger numbers for our analyses.

We hypothesize that DTaP or DTwcP vaccines, whether containing thimerosal or not, should have a similar incidence rate of adverse events. The assumption of similar reactogenicity following the vaccines under study forms the basis of our null hypothesis.

We analyzed DTaP and DTwcP vaccines by manufacturer, so that we could compare thimerosal-containing DTaP and DTwcP vaccines administered from 1992 through 2000 against thimerosal-free DTaP vaccines administered from 1997 through 2000. We used denominators obtained from the Biological Surveillance Summaries of the CDC to determine the number of doses of each manufacturer administered. Based upon this information, we were able to calculate incidence rates of adverse events following vaccination.

We are precluded from giving incidence rates, the number of doses administered, or types of DTaP or DTwcP vaccine, because this information could reveal the identities of the manufacturers and the CDC claims that this information is proprietary."

We compared the incidence rates of adverse events following thimerosal-containing DTaP and DTwcP vaccines against thimerosal-free DTaP vaccines in order to determine relative risk. The relative risk value was obtained by dividing the incidence rate of the adverse event following thimerosal-containing DTaP or DTwcP vaccines by the incidence rate of the adverse event following thimerosal-free DTaP vaccines. The relative risks of the adverse events analyzed were plotted against the amount of mercury that each child had received. By definition, since we assume that the populations under study are similar and we are tracking only the amount of mercury that children received from the thimerosal-containing or thimerosal-free vaccines under study, the initial point analyzed was zero micrograms of mercury and had a relative risk of one.

| Age (months) | Dose (micrograms) | Permissible EPA Dose (micrograms) $P_{05}$ (Ave weight in Kg) | Permissible EPA Dose (micrograms) $P_{50}$ (Ave weight in Kg) | Permissible EPA Dose (micrograms) $P_{95}$ (Ave weight in Kg) |
|---|---|---|---|---|
| 0 | 12.5 | 0.262 (2.62) | 0.330 (3.30) | 0.404 (4.04) |
| Instantaneous Relative Excess | - | 48 | 38 | 31 |
| 2 | 62.5 | 0.417 (4.17) | 0.486 (4.86) | 0.558 (5.58) |
| Instantaneous Relative Excess | - | 150 | 129 | 112 |
| 4 | 62.5 | 0.552 (5.52) | 0.654 (6.54) | 0.760 (7.60) |
| Instantaneous Relative Excess | - | 113 | 96 | 82 |
| 6 | 50 | 0.654 (6.54) | 0.780 (7.80) | 0.880 (8.80) |
| Instantaneous Relative Excess | - | 76 | 64 | 57 |
| 15 | 25 | 0.918 (9.18) | 1.05 (10.5) | 1.23 (12.3) |
| Instantaneous Relative Excess | - | 27 | 24 | 20 |
| 60 | 25 | 1.40 (14.0) | 1.86 (18.6) | 2.32 (23.2) |
| Instantaneous Relative Excess | - | 18 | 13 | 11 |

*Table 1. A summary of the instantaneous mercury exposure levels of US infants at various times as part of their childhood immunization schedule in comparison to the maximum daily EPA established limits.*

United States
Department of Education Report

The 2001 US Department of Education report was analyzed to determine the number of children at various ages who had developed various conditions.[12] The conditions analyzed included: autism, speech disorders, orthopedic impairments, visual impairments, and deaf-blindness. We determined the prevalence of each of these conditions based upon the number of births in each birth cohort as per the CDC's yearly live birth surveillance data.[13] The birth cohort years analyzed were 1984, 1985, 1990, 1991, 1992, 1993, and 1994.

We then calculated the amount of mercury that had been administered on average to each child in a birth cohort, based upon the Biologic Surveillance Summaries of the CDC. Then the prevalence of the various conditions analyzed was plotted against the amount of mercury that each child received.

**Results**

Table 1 presents a summary of the instantaneous mercury exposure of US infants at various times as part of their childhood immunization schedule in comparison to the EPA established limits. This table shows that the instantaneous relative excess mercury that US children received from their childhood immunizations ranged from 11 to 150-fold at a given age in comparison to the US EPA safety guidelines for the daily maximum oral ingestion of methylmercury. In addition, these data show that children received an instantaneous relative excess mercury doses in comparison to the FDA safety

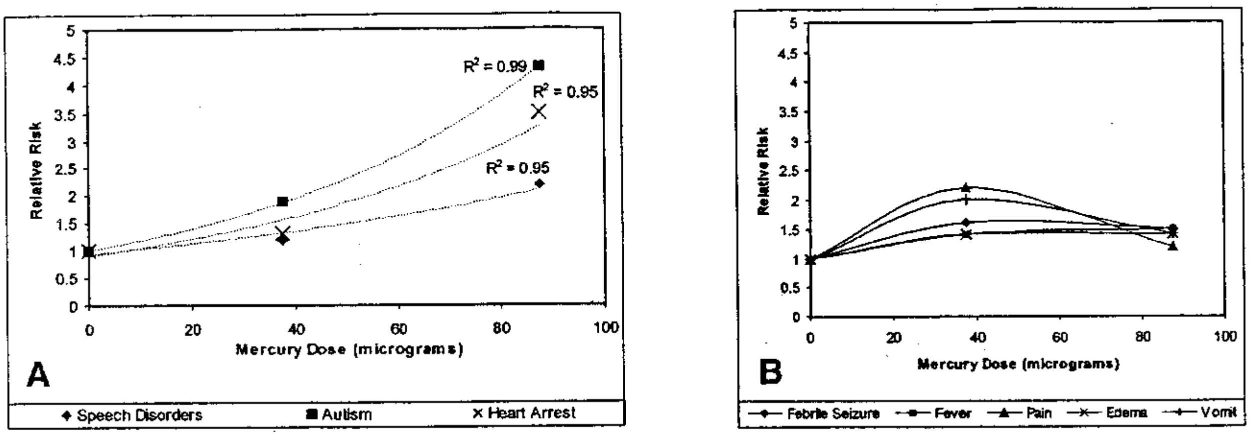



*Figure 1. **(A)** Neurodevelopment disorders and heart disease conditions reported following thimerosal-containing DTaP in comparison to thimerosal-free DTaP vaccines for increasing mercury dosage that children received from thimerosal; **(B)** Control adverse events reported acutely following thimerosal-containing DTaP in comparison to thimerosal-free DTaP vaccines for increasing mercury dosage from thimerosal*

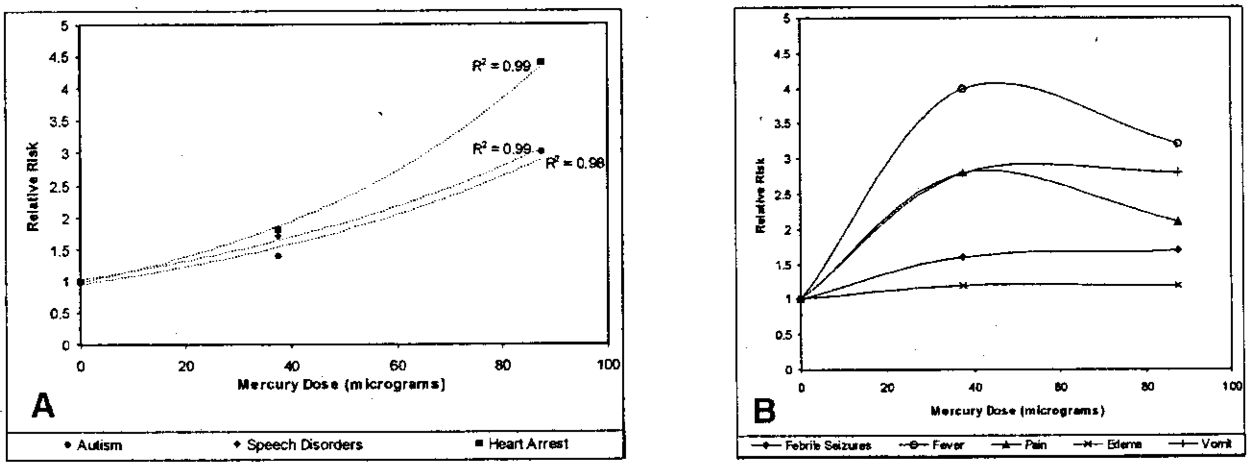



*Figure 2. **(A)** Neurodevelopment disorders and heart disease conditions reported following thimerosal-containing DTwcP in comparison to thimerosal-free DTaP vaccines for increasing mercury dosage that children received from thimerosal; **(B)** Control adverse events reported acutely following thimerosal-containing DTwcP in comparison to thimerosal-free DTaP vaccines for increasing mercury dosage from thimerosal*

guidelines for the oral ingestion of methylmercury, ranging from 2.7 to 37-fold at a given age.

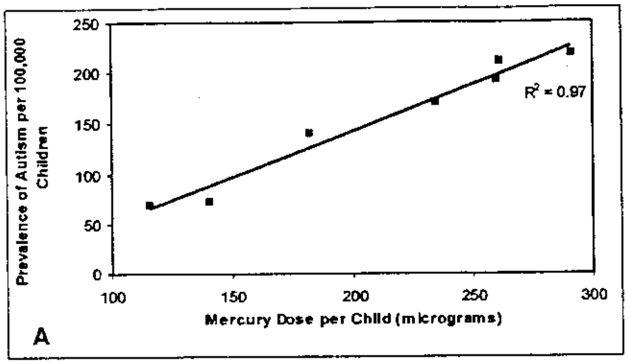
Figure 1A plots the relative risk of speech disorders, autism, and heart arrest reported after thimerosal-containing DTaP in comparison to thimerosal-free DTaP vaccines for increasing doses of mercury. We found that the data points for each condition closely followed exponential distributions.

Figure 1B plots the relative risks of febrile seizure, fever, pain, edema, and vomiting reported after thimerosal-containing DTaP in comparison to thimerosal-free DTaP vaccines. We found that administration of thimerosal-containing DTaP vaccines slightly raised the rate of adverse events compared to thimerosal-free DTaP vaccines, but the increased relative risks did not correlate with the total amount of mercury the children received.

Figure 2A shows the relative risk of speech disorders, autism, and heart arrest reported after thimerosal-containing DTwcP in comparison to thimerosal-free DTaP vaccines for increasing dosage of mercury. We found that the data points closely followed exponential distributions.

Figure 2B shows the relative risk of febrile seizure, fever, pain, edema, and vomiting after thimerosal-containing DTwcP in comparison to thimerosal-free DTaP vaccines for different mercury doses. We found that administration of thimerosal-containing DTwcP vaccines significantly raised the rate of adverse events compared to thimerosal-free DTaP vaccines, but the increased relative risks did not correlate with the total amount of mercury the children received.

Figures 3A-B show the prevalence of autism and speech disorders as a function of the mercury dose that children received from thimerosal contained in their childhood vaccines. We found that the conditions analyzed closely followed linear distributions with an increase of about one case of autism per 100,000 children for every microgram present in childhood vaccines and about one case of speech



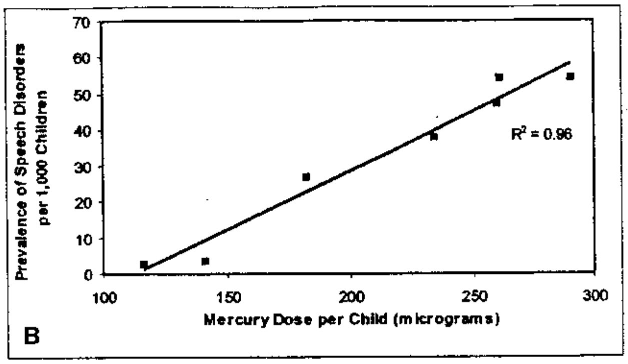
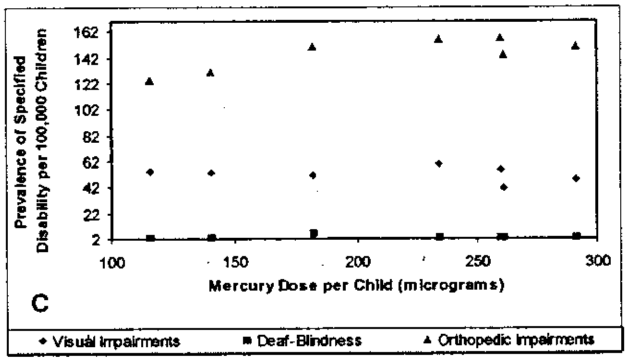




*Figure 3. (A) Autism disabilities reported in comparison to the average mercury dosage from thimerosal in childhood vaccines; (B) speech disorders reported in comparison to the average mercury dosage from thimerosal in childhood vaccines; (C) visual impairment, deaf-blindness and orthopedic impairment (control disabilities) in comparison to the average mercury dosage from thimerosal in childhood vaccines*




disorders per 1,000 children for every 3 µg of mercury present in childhood vaccines.

Figure 3C shows the prevalence of the control disabilities of visual impairment, deaf-blindness, and orthopedic impairment as a function of the mercury dose that children received from thimerosal contained in their childhood vaccines. We found that the prevalence of these conditions did not correlate with the increasing total amount of mercury the children received.

**Discussion**

It is clear from our analysis, shown in Table 1, that US infants are exposed to mercury levels from their childhood immunization schedule that far exceed the EPA and FDA-established maximum permissible levels for the daily oral ingestion of methylmercury. The fact that mercury in the vaccines is given by injection rather than by oral ingestion only makes the exposure levels worse because Geier et al. showed that the distribution of foreign particles in mice reached several-logs higher concentration in organs following intravenous or intramuscular injections than via oral ingestion.[14]

Our previous studies comparing DTaP with and without thimerosal have shown a statistically and clinically significant increase in neurodevelopment disorders in those vaccinated with thimerosal-containing vaccines.[8] Our current study not only shows that those vaccinated with thimerosal-containing DTaP and DTwcP have higher rates of speech disorders, autism, and heart arrest overall, but also that the relative risk of each of these disorders correlated with increasing doses of mercury contained in childhood vaccines, as illustrated in Figures 1A and 2A. Figures 1B and 2B show that exposure to increasing doses of mercury is not correlated to acute vaccine adverse events including febrile seizures, fever, pain, edema, or vomiting.

Our demonstration of a significant overall increase in the relative risks of acute adverse events following DTwcP vaccine when compared to thimerosal-free DTaP vaccine is not surprising since our previous studies have shown that DTwcP vaccines are far more reactogenic than DTaP vaccines.[15,16] However, we observed that only those events for which causation by thimerosal is biologically plausible were found to be correlated with the mercury levels children received in their vaccines.

Our analyses of a completely independent source, the US Department of Education's report on the prevalence of various childhood disease among school children of various ages, showed autism and speech disorders were correlated with increasing mercury from childhood vaccines, as shown in Figures 3A-B. No correlation was seen between increasing mercury exposure from childhood vaccines and the prevalence of visual impairments, deafness-blindness, or orthopedic impairments, as shown in Figure 3C.

The lack of correlation between acute events and increasing mercury exposure levels in the VAERS data argues against reporting bias or differences in the vaccines themselves and argues for the specific effects of thimerosal on neurodevelopment disorders and heart disease. Likewise, the lack of correlation between visual impairments, deaf-blindness, or orthopedic impairments and the increasing mercury exposure levels in the US Department of Education data also argues for the specific effects of thimerosal in childhood vaccines on the prevalence of autism and speech disorders.

As an additional epidemiological confirmation of our findings, we analyzed the CDC's Phase I Thimerosal Vaccine Safety Datalink (VSD) data.[17] We found this data showed that the increasing relative risk of developmental neurologic disorder, autism, speech disorder, and attention deficit disorder all closely followed exponential distributions with increasing mercury levels from the thimerosal that children received as part of their childhood immunizations.

We conducted a MEDLINE (1966-2003) search for the terms merthiolate and thimerosal and found almost 1,500 references, primarily about various adverse outcomes following exposure. Of particular interest, Bernard et al. have compared the similar biological abnormalities commonly found in autism and the corresponding pathologies arising from mercury exposure.[18] Distinct similarities were found between autism and mercury exposure in their effects upon biochemistry, the immune system, the central nervous system structure, neurochemistry, and neurophysiology.

A study performed by Magos et al. in rats compared the effects of the administration of similar doses of ethylmercury and methylmercury.[19] They found that higher concentrations of inorganic mercury in the kidneys and brain were present in ethylmercury-treated rats compared to methylmercury-treated rats. They determined that there was little difference in the neurotoxicity found in ethylmercury and methylmercury-treated rats when effects on the dorsal root ganglia or coordination disorders were compared. The authors also determined that microgram quantities of organic mercury alone in the rat brain were in some cases associated with neurotoxicity, indicating that the presence of inorganic mercury was not necessary for neurotoxicity.

It has been reported that administration of thimerosal results in the immediate release of ethylmercury to the surrounding tissues.[20] The reason for this stems from the fact that thimerosal contains the ethylmercury radical attached to the sulfur atom of the thiol group of salicylic acid. Generally, mercuric ions bind tightly but reversibly to thiol ligands.[21] It is likely, therefore, that the ethylmercury cation will dissociate from the thiosalicylic acid moiety immediately after injection to bind to the surrounding thiol ligands present in great excess in tissue proteins.[28]

Rapid deposition of ethylmercury in tissues following administration of thimerosal from vaccines is suggested by a recent publication by Pichichero et al.[22] The authors examined the concentrations of mercury in the blood, urine, and stool from 3 to 28 days following thimerosal-containing vaccines in 40 full-term infants aged 6 months and younger in comparison to 21 control infants receiving thimerosal-free vaccines. The mean mercury doses of the infants exposed to thimerosal were 45.6 µg (range 37.5-62.5) for 2-month-olds and 111.3 µg (range 87.5-175.0) for 6 month-olds. Blood mercury in thimerosal-exposed 2-month-old infants ranged from less than 3.75 to 20.55 nmol/L; in 6 month-old infants all values were lower than 7.50 nmol/L. Only 15 blood samples from controls contained quantifiable mercury. Concentrations of mercury were low in the urine after vaccination but were high in the stools of thimerosal-exposed 2-month-old infants (mean 82 ng/g dry weight) and in 6-month-old infants (mean 58 ng/g dry weight).

The authors estimated that the blood half-life of ethylmercury was 7 days (95% confidence interval of 4 to 10 days). The study was unable to determine the ultimate disposition of most of the mercury with which infants were injected. However, it has been determined that uptake of mercury in the brain is 5to 7 times greater than in the blood.[28,9] Therefore, because of the similar theoretical and experimental toxicities of ethylmercury and methylmercury, and the immediate buildup of ethylmercury from thimerosal in the tissues of the body, especially the preferential buildup in the brain, there appears to be good biologic plausibility for the neurodevelopment disorders and heart conditions observed in this study.

On July 7, 1999, the American Academy of Pediatrics and the US Public Health Service issued a joint statement calling for the removal of thimerosal from vaccines, prompted by a risk assessment from the FDA.[9] The 2001 IOM report stated that technology is available to manufacturers in the US to allow for the removal of thimerosal from childhood vaccines in a timely manner and that only a small number of thimerosal-containing vials remain on the shelf.[7]

We have recently reviewed the 2003 US Physician's Desk Reference (PDR) and found that some childhood vaccines still contain thimerosal. DTaP manufactured by Aventis Pasteur contains 25µg of mercury, *Hemophilus influenza* b (Hib) vaccine manufactured by Wyeth contains 25 µg of mercury, and pediatric hepatitis B vaccine manufactured by Merck contains 12.5 µg of mercury.[24] In addition, influenza vaccine that is recommended for an increasing segment of the pediatric population in the US also contains 25 µg of mercury. Therefore, it is indeed possible that children in the US in 2003 may be exposed to levels of mercury from thimerosal contained in their childhood vaccinations that are at a higher level than at any time in the past. Possible total childhood mercury in 2003 is more than 300 µg.

Because of the data implicating thimerosal levels with increasing rates of autism, speech disorders, and heart disease, it would seem prudent to completely remove thimerosal from all childhood vaccines immediately. The use of single-dose vials would alleviate the need for any preservative in the vaccines.

Parents should be encouraged to avoid exposing their children to additional mercury from sources other than vaccines. Until recently, Rh-negative women were routinely given Rhogam injections, which contained significant amounts of thimerosal, several times during their pregnancies. Fortunately, thimerosal has been removed from Rhogam. Pregnant women and children should avoid eating seafood that may contain significant quantities of mercury. The mean mercury levels in various seafood species have been determined by the Center for Food Safety and Applied Nature Section of the FDA.[25] The FDA currently recommends that pregnant women and those women who may become pregnant avoid species with the highest average amounts of methylmercury and that a "balanced" diet of seafood consumption should be followed so as to keep methylmercury levels low.[26] Additionally, fetuses may be exposed to mercury from the amalgam used in their mother's fillings. Also, patients should be made aware that there are other prescription drugs and over-the-counter medications that contain significant amounts of thimerosal.[24]

These other sources of mercury, while potentially significant, probably had a limited effect on the results of this study because the populations analyzed were large and there should have been equal exposure to other sources of mercury among the populations examined.

## Conclusion

This study provides strong epidemiological evidence for a link between increasing mercury from thimerosal-containing childhood vaccines and neurodevelopment disorders and heart disease. In light of voluminous literature supporting the biologic mechanisms for mercury-induced adverse reactions, the presence of amounts of mercury in thimerosal-containing childhood vaccines exceeding Federal Safety Guidelines for the oral ingestion of mercury, and previous epidemiological studies showing adverse reactions from such vaccines, a causal relationship between thimerosal-containing childhood vaccines and neurodevelopment disorders and heart disease appears to be confirmed. It is to be hoped that complete removal of thimerosal from all childhood vaccines will help to stem the tragic, apparently iatrogenic epidemic of autism and speech disorders that the United States is now facing.

Dr. Mark Geier has done consulting work and appeared as an expert witness, and David Geier has done consulting worki in cases before the National Vaccine Injury Compensation Program (NVCIP) and in civil suits involving vaccine adverse reactions. To date, none of these cases have involved thimerosal.

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## REFERENCES

[1] Burd L, Fisher W, Kerbeshian J. A prevalence study of pervasive developmental disorders in North Dakota. *J Am Acad Child Adolesc Psychiatry* 1987;26:700-703.

[2] Ritvo ER, Freeman BJ, Pingree C, et al. The UCLA-University of Utah epidemiologic survey of autism: Prevalence. *Am J Psychiatry* 1989;146:194-199.

[3] Yeargin-Allsopp M, Rice C, Karapurkar T, et al. Prevalence of autism in a US metropolitan area. *JAMA* 2003;289:49-55.

[4] Guallar E, Sanz-Gallardo MI, van't Veer P, et al. Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med* 2002;347:1747-1757.

[5] Salonen JT, Seppanen K, Nyyssonen K, et al. Intake of mercury from fish, lipid peroxidation and risk of myocardial infarction and coronary cardiovascular and any death in Eastern Finnish men. *Circulation* 1995;91:645-655.

[6] Salonen JT, Seppanen K, Lakka TA, et al. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population-based prospective 4-year followup study in men in eastern Finland. *Atherosclerosis* 2000;148(2):265-273.

[7] Institute of Medicine (US). *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders*. Washington, DC: National Academy Press; 2001.

[8] Geier MR, Geier DA. Neurodevelopmental disorders following thimerosal-containing vaccines. *Exp Biol Med*. (in press).

[9] Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics* 2001;107:1147-1154.

[10] Diem K, Lentner C (eds). *Geigy Scientific Tables*. Ardsley, NY: Geigy Pharmaceuticals, 1974.

[11] Niu MT, Rhodes P, Salive M, Lively T, Davis DM, Black S, Shinefield H, Chen RT, Ellenberg SS. Comparative safety of two recombinant hepatitis B vaccines in children: Data from the Vaccine Adverse Events Reporting System (VAERS) and Vaccine Safety Datalink (VSD). *J Clin Epidemiol* 1998;51:503-510.

[12] Department of Education (US). Annual report to Congress on the Implementation of the individuals with Disabilities Education Act, 23rd annual report. Washington, DC: Office of Special Education Programs, A-14 (2001).

[13] Live births by age of mother and race: United States, 1933-98. http://www.cdc.gov/nchs/data/natality/mage33tr.pdf (Accessed January 1, 2003).

[14] Geier MR, Trigg ME, Merril MR. Fate of bacteriophage in non-immune germ-free mice. *Nature* 1973; 246: 221-222.

[15] Geier DA, Geier MR. Clinical implications of endotoxin concentrations in vaccines. *Ann Pharmacother* 2002;36: 776-780.

[16] Geier DA, Geier MR. Serious neurological conditions following pertussis immunization: an analysis of endotoxin levels, the Vaccine Adverse Events Reporting System (VAERS) Database and Literature Review. *Pediatr Rehabil* 2002; 5: 177-182.

[17] Verstraeten T, Davis R, DeStefano F. Thimerosal VSD study phase I (2/29/00). Obtained via Freedom of Information Act request.

[18] Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: A novel form of mercury poising. *Med Hypothesis* 2001; 56: 462-471.

[19] Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. The comparative toxicology of ethyl- and methylmercury. *Arch Toxicol* 1985;57: 260-267.

[20] Clarkson TW. The three faces of mercury. *Environ Health Perspect* 2002;110(Suppl 1):11-23.

[21] Carty AJ, Malone SF. The chemistry of mercury in biological systems. In: *The Biogeochemistry of Mercury in the Environment*. Nriagu JO (ed). New York: Elsevier/North Holland; 1979:433-459.

[22] Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: A descriptive study. *Lancet* 2002;360:1737-1741.

[23] Cernichiari E, Brewer R, Myers GJ, et al. Monitoring methylmercury during pregnancy: Maternal hair predicts fetal brain exposure. *Neurotoxicology* 1995;16(4):705-710.

[24] Physician Desk Reference (PDR). Montvale, NJ: Medical Economics Company; 2003: 790-795,811-816,1554-1559.

[25] Mercury levels in seafood species. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, May 2001. Accessible at: http://www.cfsan.fda.gov/~frf/sea-mehg.html (Accessed January 1, 2003).

[26] Bolger P, Schwetz BA. Mercury and health. *N Engl J Med* 2002;347:1735-6.

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# Reduced Levels of Mercury in First Baby Haircuts of Autistic Children

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**Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.**

Keywords Amalgam, Autism, Hair, Mercury, Thimerosal

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Autism has been defined by symptoms rather than causes since it was first characterized by Kanner in the 1940s (Eisenberg and Kanner 1956). Since Rutter's (Rutter 1978) further elaboration of diagnostic standards in 1976, the prevailing standards for diagnosis (*Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition [DSM III] 1980; 3rd edition—revised [DSM-III-R] 1987; 4th edition [DSM IV] 1994) have included impairment in three domains: social relatedness, communication, and behavior. In a small number of cases, either genetic (Wahlstrom et al. 1986; Bolton et al. 2002; Steffenburg et al. 1996) or environmental (Stromland et al. 1994; Williams and Hersh 1997; Aronson, Hagberg, and Gillberg 1997) causes have been established, but the vast majority of cases remain idiopathic.

The need to account for the relative contribution of genetic and environmental causes has taken on increased importance in light of possible sharp increases in the incidence of autism. Early prevalence studies in the United States (Burd, Fisher, and Kerbeshian 1987; Treffert 1970; Ritvo et al. 1989) and the United Kingdom (Lotter 1966; Wing and Gould 1979; Deb and Prasad 1994) reported low rates of autism—generally less than 5 per 10,000—among children born before 1990. Studies of populations born in the 1990s, however, show far higher (Bertrand et al. 2001; Baird et al. 2000) and increasing (Department of Developmental Services 1999; Kaye, del Melero-Montes, and Jick 2001; Taylor et al. 1999) rates of autism and autism spectrum disorders (ASDs), in some cohorts as high as 55 per 10,000 for autism and 80 per 10,000 for ASDs.

These increases clearly point to the rising importance of environmental factors and raise the possibility of an etiological role for toxic exposures: either prenatal, postnatal, or in some cumulative pattern that combines the effect of maternal, gestational, and infant exposures. One group (Bernard et al. 2001) has hypothesized a causal connection between mercury exposure and the symptoms of autism.

Until recently, thimerosal, a preservative containing 49.6% ethyl mercury, was used in three childhood vaccines: hepatitis B, *Haemophilus influenzae B* (Hib), and diphtheria-pertussis-tetanus (DPT). Hib and hepatitis B were introduced to the U.S.

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infant vaccination schedule in October 1990 and November 1991, respectively. In addition, most varieties of Rho D immunoglobulin injections, administered to Rh-negative mothers during pregnancy, contained thimerosal until late in the 1990s. The Institute of Medicine has investigated the connection (Stratton, Gable, and McCormick 2001) between mercury exposure from thimerosal-containing vaccines and neurodevelopmental disorders, including autism, and found insufficient evidence to accept or reject a causal connection, but concluded that such a connection was biologically plausible and recommended a comprehensive research program.

In addition to ethyl mercury, other possible sources of early mercury exposure include fetal exposures to inorganic mercury inhaled by the mother from dental amalgam fillings (Drasch et al. 1994; Vimy et al. 1997) and to methyl mercury intestinally absorbed as a consequence of maternal fish consumption.

Little is known about the specific patterns of mercury absorption, distribution, metabolism, or excretion in human infants. The large majority of infants immunized with the full complement of thimerosal-containing vaccines have not been diagnosed with an adverse effect, such as neurodevelopmental delay. Nevertheless, ecological analysis of the timing of the increases in autism incidence and the increased exposure to mercury in thimerosal-containing vaccines fails to exclude a causal relationship between the two trends of rising autism incidence and rising mercury exposure (Blaxill 2001).

Fully prospective studies of the role of mercury exposure in autism have not yet been designed and even retrospective studies are highly constrained by availability of relevant biological samples. Many families do, however, retain locks of infant hair, especially the first baby haircut. These samples provide an opportunity for analysis when other opportunities have passed. Although hair mercury levels provide only a partial insight into the excretion patterns of autistic infants, they offer substantial availability advantages and can provide a useful test of the plausibility of the autism-mercury hypothesis.

In a clinical practice, one of the study authors (ASH) submitted hair samples from autistic patients for commercial laboratory testing for toxic metal exposure. Most of these mercury hair levels were found to be low, contrary to a first-order hypothesis of heavy metal toxicity in autism. She then asked patients to submit first baby haircut samples for analysis, thereby testing a sample that would more accurately reflect early exposures. With two exceptions (these coming from a different commercial laboratory than her preferred source and the source used in the current study), these samples yielded hair mercury levels that were consistently close to zero.

Based on this observation, and on the possibility that impaired mercury excretion might be an important susceptibility factor underlying recent increases in autism, she expanded her investigation. She increased the sample of autistic first baby haircut samples and collected a set of age- and gender-matched control baby haircut samples. Notably, the control samples were collected under the condition that the child received all their childhood vaccinations on schedule, in order that they would show comparable postnatal exposure levels. Consequently, this study does not attempt to examine the role of childhood vaccine exposures in autism. Although there are limits to the design, we believe that our study effectively examines the null hypothesis of no differential excretion rates in the hair of infants subsequently diagnosed with autism.

## MATERIALS AND METHODS

### Patient Recruitment and Profile (Table 1)

All autistic patients were referred to the clinical practice of ASH with a confirmed diagnosis of DSM IV autism by either a pediatric neurologist or developmental pediatrician. The mother of each autistic child was interviewed for exposure information using a structured survey questionnaire. The autistic children were between the ages of 2 and 15 at the time of interview, with a median age of 7. Although the location of primary residence was slightly skewed to the Midwest and Southeast, in part due to proximity to the clinical practice, the autistic patients provided a good cross-section of the different regions of the United States, with an additional 6% coming from England, Canada, and Mexico. Boys outnumbered girls, with a male:female ratio of 3.5:1, consistent with the typical population prevalence in autism studies (Fombonne 1999).

### Conditions of Autistic Baby Hair Collection

Autistic patients sent their baby hair samples directly to Doctor's Data Inc. (DDI, West Chicago, IL) following DDI's instructions for the hair minerals test. First baby haircut samples had

TABLE 1
Study group profiles

| | Autistic group | Control group |
|---|---|---|
| Number of males/ females (ratio) | 73/21 (3.5:1) | 34/11 (3.1:1) |
| Median year of birth (range) | 1994 (1985–1999) | 1994 (1990–1999) |
| Median months at baby haircut timing (range) | 17.7 (11–24) | 17.8 (12–24) |
| Residence[a] | | |
| Northeast | 15% | 22% |
| Midwest | 28% | 22% |
| Southeast | 25% | 22% |
| Mountain/Plains/ South Central | 14% | 20% |
| West | 12 % | 13% |
| International | 6% | 0% |

[a]Northeast: CT, MA, VT, NY, NJ, PA. Midwest: IL, OH, MI, WI, MN, MO. Southeast: FL, GA, NC, SC, VA, LA, MS, AL, AR, KY, TN. Mountain/Plains/South Central: CO, KS, ND, SD, NE, UT, ID, TX, OK, AZ. West: CA, WA, NV. International: Canada, Mexico, England.

been collected by the parents between 11 and 24 months of age, with a mean age at haircut of 17.7 months. The minimum sample amount was 0.25 g. Before deciding to standardize on a single testing source, a minority of hair samples (20 in all) were collected in the course of clinical treatment of additional autistic patients and sent to a separate commercial laboratory—not DDI—that performs similar testing. These results were reviewed by the clinic but excluded from this study. Results from this other laboratory were similar to those from DDI, showing low hair mercury levels in autistic patients. Two of the 20 excluded test results included mercury levels that were higher than the reported levels for any of the autistic subjects in the present study.

### Controls Recruitment and Baby Hair Collection

Normal controls were recruited through an appeal to autism parent groups and through autism newsletters. None of these control children or parents were interviewed in person, but each of the mothers was interviewed over the telephone. Hair collection procedures were the same as for the autistic patients. The inclusion criteria for controls included the following: no developmental disabilities or chronic illness of any kind, no siblings on the autistic spectrum, and completion of the recommended childhood vaccinations on schedule. These controls were recruited with the objective of matching the autistic patients in terms of gender and age profile (see Table 1). Although not a condition of recruitment, the state of residence was quite similar to the autistic sample, minimizing the possibility of regional exposure bias.

### Hair Analysis Methods

Laboratory testing was conducted using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) in blinded fashion to the clinical status of the hair provider. Hair specimens were collected based on retained samples of a minimum required amount of 0.25 g of hair from the child's first haircut. In the laboratory, the hair specimens were further cut and washed using a modified method developed by the International Atomic Energy Agency (IAEA) (Ryabukin 1998). Aliquots (about 0.2 g) of the washed hair samples were digested with nitric acid in a CEM (CEM Corporation, Matthews, NC, USA) microwave oven with temperature feedback control. All element determinations were made on an ICP-MS (Elan 5000; Perkin-Elmer, Norwalk, CT, USA) using a flow injection sample uptake system (FIAS 400; Perkin-Elmer). Accuracy was assessed and verified using a hair standard reference material (SRM) from China (GBW 09101, 30 elements). Puchyr et al. (1998) have described this method and analytical performance in detail.

### Clinical Observation of Subjects Based on Clinic Visits

All previously diagnosed autistic patients were also observed by ASH in a clinical setting. A repetitive diagnostic interview was not conducted, but an overall assessment of each child was performed and each was assigned an autism severity level: severe, moderate, or mild. The definitions for these categories were as follows: (a) severe—no expressive language at all, very little evidence of receptive language, constant divergent gaze in presence of clinician or parent, no toy play, "people treated as objects"; (b) mild—some expressive language, including short phrase speech and ability to communicate wants and needs, responsive to commands indicating functional receptive language, some eye contact, some appropriate toy play, obvious connection with parents and/or other family members; and (c) moderate—subjects not meeting criteria for either the severe or mild groups.

### Data Collection for Other Maternal Exposures

ASH interviewed the mothers of autistic and normal children to obtain information on mercury exposure during and after gestation. Exposure measures were developed from survey questions in four categories: (a) maternal amalgams during pregnancy were estimated by direct observation by the mother (either using a mirror, or counted by her husband) of amalgam surfaces at time of interview less new fillings since the gestation period; (b) exposures through Rho D immunoglobulin injections during pregnancy were self-reported by the mother; (c) childhood vaccinations, including the timing of exposures to hepatitis B, DPT, and Hib vaccines, were obtained based on a joint review of the child's pediatrician's records; and (d) fish consumption during pregnancy in four categories was estimated using a four-level scale. The four levels estimated were based on the relative frequency of meals in which fish was consumed: "heavy" was once a week or more, "moderate" was less than weekly and more than monthly, "little" was less than once a month, with the final category being "none."

### Statistical Analysis

Data were analyzed using Microsoft Excel version 9.0.3821 SR-1. Comparison of distributions of autistics and controls was made using the two-tailed test. Multiple-regression analysis on the normal hair sample was performed with the hair mercury level as the dependent variable and three independent variables. For amalgam fillings, we used the square of the number of fillings. We chose an exponential curve for several reasons: mothers with more fillings would be likely to have larger fillings and larger exposed surface areas per filling; multiple amalgams would be more likely to react with each other and release higher levels of mercury due to galvanism or abrasion; higher levels of amalgam exposure could lead to greater retention in maternal tissue; and mothers with large numbers of fillings would be more likely to have had recent dental work done. In addition, the exponential relationship produced consistently superior statistical correlations. For fish diets, we assigned a monthly number of fish meals for each response level: 5 per month for "heavy" consumption, 2.5 per month for "moderate" consumption, 0.5 for "little" consumption, and 0 for no consumption. For vaccine exposure,

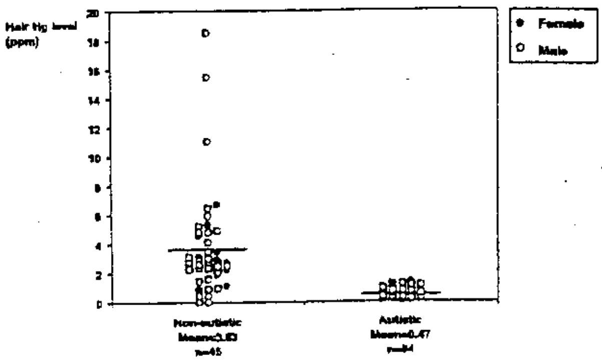



FIGURE 1

A plot of the birth hair mercury levels of nonautistic versus autistic children. Solid circles represent individual female subjects and open circles represent individual male subjects.

we used the total number of micrograms received through all thimerosal-containing vaccines.

## ANALYSIS OF DATA AND RESULTS

### Hair Mercury: Autistics Versus Controls

Figure 1 shows the results of the analysis comparing the excretion of mercury in baby hair of autistic children and normal controls. The hair levels of mercury in autistic children were significantly lower than in controls ($p < .0000004$), with a mean of 0.47 ppm compared to a mean of 3.63 ppm in the control group.

### Rho D Immunoglobulin and Amalgam Exposure: Autistics Versus Controls

Despite the lower levels of mercury excretion in baby hair, the mothers of autistic children had higher exposures to mercury when pregnant with their autistic child than did normal controls. Table 2 shows that the number of Rho D immunoglobulin injections received by mothers in the autistic group was significantly higher than the mothers of controls (0.52 versus 0.09; $p < .0000004$). Forty-six percent of the autistic mother received Rho D immunoglobulin injections as compared to 9% of the control mothers. In addition, the number of amalgam fillings present in the mouths of mothers of autistic children exceeded the number of fillings for mothers of controls (8.35 versus 6.60; $p < .01$). Thirty-four percent of the mothers in the autistic sample had 10 or more amalgam fillings as compared to 18% of the controls.

### Hair Mercury Levels Within Autistic Population: Mild Versus Moderate Versus Severe

Within the autistic sample, the level of mercury in hair was inversely correlated with the symptom severity level. Figure 2 displays the distribution of hair levels across the three subgroups of autistic children. The mean hair mercury content was 0.21 ppm in the severe group, 0.46 ppm in the moderate group (severe versus moderate: $p < .000002$), and 0.71 ppm in the mild group (moderate versus mild: $p < .0004$; severe versus mild: $p < .00000003$). Even these stark differences were somewhat moderated by a clear trend toward reduced mercury levels in female hair within the mild group.

TABLE 2

Exposure differences in autistic group as compared to controls

| | Autistic group ($N = 94$) | Control group ($N = 45$) |
|---|---|---|
| Mercury levels in first baby haircut (ppm, mean ± *SD*) | 0.47 (±0.28)[a] | 3.63 (±3.56) |
| Rho D immunoglobulin shots during pregnancy (number per mother, mean ± *SD*) | 0.53 (±0.67)[b] | 0.09 (±0.29) |
| Amalgam fillings during pregnancy (number per mother, mean ± *SD*) | 8.35 (±3.43)[c] | 6.60 (±3.55) |

[a]Statistically different from control group ($p < .0000004$).
[b]Statistically different from control group ($p < .0000004$).
[c]Statistically different from control group ($p < .01$).

### Differences Within Autistic Population: Gender and Developmental Patterns

Table 3 provides information on other differences between the three subgroups within the autistic sample. In addition to the differences in hair mercury levels, the gender distribution varied substantially across the three subgroups. The mild group had the highest percentage of females, at 56%, whereas the moderate and severe groups had 14% and 4% females, respectively. In Figure 2, it is also apparent that the female children in the mild

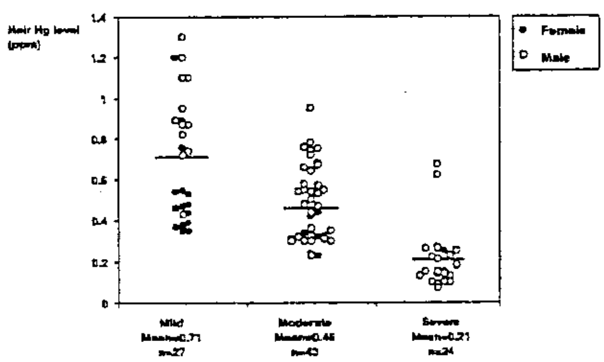



FIGURE 2

A plot of the birth hair mercury levels in autistic children based on the clinical severity of the disease. Solid circles represent individual female subjects and open circles represent individual male subjects.

TABLE 3
Differences within autistic population

| | Mild group ($N = 27$) | Moderate group ($N = 43$) | Severe group ($N = 24$) |
|---|---|---|---|
| Mercury levels in first baby haircut (ppm, mean ± *SD*) | 0.71 ppm (±0.3) | 0.46 ppm (±0.19)[a] | 0.21 ppm (±0.18)[a,b] |
| Males: females | 12:15 | 37:6 | 23:1 |
| Percent regressive | 100 | 93 | 21 |

[a]Statistically different from mildly autistic group ($p < .0004$).
[b]Statistically different from mildly autistic group ($p < .000000003$).
[c]Statistically different from moderately autistic group ($p < .0000002$).

group made up over 90% of the children below the mean and only 21% of the children above the mean. In addition, the developmental patterns varied strongly across the three subgroups. The severe group was the most likely to have demonstrated consistency in symptoms from birth, only 21% displayed any pattern of developmental regression. By contrast, the vast majority of the mild and moderate groups reported some kind of developmental regression.

### Correlation Between Exposure and Hair Levels

In the control sample, the levels of mercury in baby hair were significantly explained by gestational mercury exposures. Figure 3 demonstrates that a single exposure variable, maternal amalgam fillings, was strongly correlated with mercury hair levels in control children, but not in autistic children. Several different regression models were applied—including one, two, and three independent variable regressions—and the three-variable equation shown in Figure 4 provided the best statistical fit. Maternal amalgam fillings were significantly correlated with mercury levels in all regressions and on their own explain over 60% of the difference in normal hair levels. Reported maternal fish consumption during pregnancy is an additional and significant contributor to hair mercury levels. Vaccine exposure from all childhood immunizations also reached significance at the 95% confidence level. By contrast, similar regressions for autistic mercury hair levels (not shown) fail to reach significance for any exposure variable. Moreover, applying the exposure coefficients from the control group to the autistic group yields a sharply higher rate of predicted excretion levels than the actual results, whereas the predicted results based on known exposures in the control group are remarkably close to the actual results (see Figure 4). This reflects the high explanatory power ($R^2 = .79$) of the multiple regression model in the control sample.

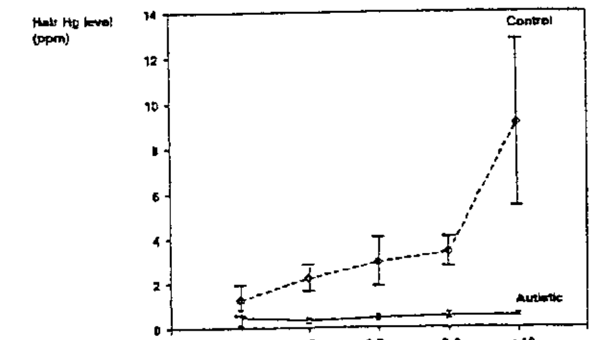


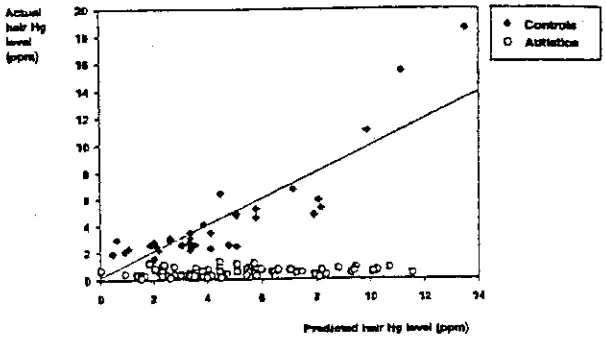

FIGURE 3
A plot of the birth hair mercury levels of nonautistic (control) children versus autistics compared to the grouped numbers of dental amalgams of the birth mothers. *N* equals the number of subjects and the control-to-autistic ratio for each subset is presented.

## DISCUSSION

### Past Studies Using Hair as a Marker in Autism

Although this is the first analysis of the first baby haircut of autistic infants, a number of previous studies have measured the hair contents of autistic subjects. The earliest studies (Wecker et al. 1985; Shearer et al. 1982; Gentile et al. 1983) analyzed hair from subjects born before 1981 and only one of these (Wecker et al. 1985) measured mercury. All of these studies found some significant differences between autistic and control groups. A group of autistic subjects averaging 5.7 years of age (Wecker et al. 1985) showed low hair levels of calcium, magnesium, copper, manganese, chromium, and lithium, but similar levels of mercury compared to controls. A group averaging 8 years of age (Shearer et al. 1982) showed low levels of cadmium excretion. A third group of unspecified age (Gentile et al. 1983) showed elevated levels of magnesium and potassium.

A more recent study (Holloway et al. 2001) of 50 autistic families (no age was specified) investigated both heavy metal exposures and hair levels for the purpose of testing the hypothesis of different metabolism and/or exposure levels between autistic




(1) Statistically significant (P<0.000000002)
(2) Statistically significant (P<0.00002)
(3) Statistically significant (P<0.02)

FIGURE 4

Actual hair levels in autistics and controls are compared to a predicted value. The predicted value is obtained using the regression equation for controls: Birth hair mercury level = (5.60) + 0.04 (amalgam volume[(1)]) + 1.15 (fish consumption[(2)]) + 0.03 (vaccine[(3)]) $R^2 = .79$. Perfect prediction of actual hair levels by the regression model is represented by the dashed line. Filled diamonds represent individual nonautistic subjects and open circles represent autistics.

subjects and controls. This study used current, not baby hair, samples and found slightly reduced levels of mercury and lead in autistic hair relative to controls and significant differences in exposure, including increased maternal intake of fish and an increased rate of ear infection in infancy and associated exposure to antibiotic treatments.

Hair analysis has frequently been used as a measure of mercury exposure. In particular, it has been common practice (Grandjean et al. 1997, 1998) to measure maternal hair levels as a marker for mother-to-fetus exposures that could affect subsequent brain development. Hair mercury analysis has also been criticized as a diagnostic tool for treatment (Kales and Goldman 2002), and hair minerals test results from commercial laboratories have been criticized as inconsistent and unreliable. A recent critical review (Seidel et al. 2001) included the testing source for this study.

In our view, this recent review offered criticisms that were applied in an undifferentiated fashion to a group of laboratories and made no attempt to distinguish between proper and improper practices within the group. Much of the criticism was justified in the case of other facilities, because many of the laboratories examined made use of outdated technologies and exploited their testing results for commercial purposes, including promotion of nutritional supplements. Close reading of the analysis shows that the laboratory used in our analysis used none of the questionable practices deployed by most other laboratories and was one of only two laboratories that employ the most advanced (ICP-MS) testing equipment. We believe that the exemplary practices of DDI, the advantages of ICP-MS, the specificity of the timing of our sample collection, the fact that the laboratory was blind to the clinical status of the samples, and our use of a single, consistently calibrated laboratory protocol all combine to mitigate any concerns over the reliability of our results.

## Infant Mercury Exposure and Autism

The mercury exposure levels in infants from the recommended U.S. childhood immunization schedule exceeded the threshold set by the U.S. Environmental Protection Agency (EPA) for most of the 1990s. This increased level of exposure came about as a consequence of the addition of two new thimerosal-containing vaccines, hepatitis B in November 1991 and Hib in October 1990, to the U.S. infant vaccination schedule. For example, a typical 2-month-old infant would have received an average of 0.25 $\mu$g/kg/day if immunized on schedule, as compared with the EPA threshold safety level of 0.1 $\mu$g/kg/day. This average exposure may well understate the severity of these exposures. On the days of vaccination, the bolus dose of mercury was many times the threshold. This excess exposure went undetected until the summer of 1999 when an Food and Drug Administration (FDA) review identified the problem. Subsequent to this discovery, the American Academy of Pediatrics (AAP) and the Centers for Disease Control and Prevention (CDC) (1999) issued a joint statement suspending the birth dose of hepatitis B vaccine and recommending the phasing out of thimerosal from all infant vaccines.

The special case of direct human infant exposure to thimerosal has never been studied. Yet the hypothesized (Bernard et al. 2001) relationship between mercury exposure and autism is supported by a number of ecological connections: increases in autism rates in the United States have accompanied increasing exposure to thimerosal-containing vaccines (Blaxill 2001); increases in autism rates in England (Lotter 1966, Wing and Gould 1979; Deb and Prasad 1994; Baird et al. 2000; Kaye et al. 2001; Taylor et al. 1999) followed closely on the heels of a change in the recommended immunization schedule and physician incentive structure (Salisbury and Dittman 1999) that increased the level of thimerosal exposure in the first four months of life; and rates of autism rose in Japan's Fukushima prefecture (Hoshino et al. 1982) immediately after the 1965 incident in neighboring Niigata prefecture when an chemical factory released large amounts of mercury into a local river.

Iatrogenic exposure to mercury has been shown to cause childhood disease. Mercury used in teething powder preparations in the first half of the 20th century was identified as the cause of acrodynia, a serious disease of young children that puzzled medical professionals for decades. Resistance to the evidence of mercury poisoning delayed full acceptance of the evidence for many years (Dally 1997). There are numerous parallels between the symptoms of acrodynia and autism, including loss of sociability and communication skills (Bernard et al. 2001).

## Animal Models of Infant Mercury Excretion

Despite the absence of direct observation of mercury toxicokinetics in human infants, there are a number of animal models that provide insight into the differences in the kinetics of mercury between infants and adults. Independent of the effects on the brain, these models demonstrate that several factors unique to infants can contribute to reduced excretion capacity.

Mercury is excreted mostly through the feces and is heavily dependent on the biliary secretion of inorganic mercury transported into bile by glutathione. Studies in neonatal rats (Ballatori and Clarkson 1984) have demonstrated reduced bile flow and glutathione excretion that together contribute to a reduced ability in infants to excrete methyl mercury. In a range of studies, research has demonstrated that both milk diets and antibiotic administration reduce the excretion of mercury (Kostial et al. 1979, 1981; Rowland, Robinson, and Doherty 1984), effects that are both common and amplified (Kostial et al. 1978) in suckling animals. The presence of healthy gut flora is critical (Rowland, Davis, and Grass 1978; Rowland, Robinson, and Doherty 1984) to the excretion of mercury and early exposure to mercury has even been shown to alter the composition of gut flora and promote both antibiotic-resistant and mercury-resistant strains (Summers et al. 1993). All of these mechanisms suggest that the infant period is one of both high potential variation in mercury excretion capacity and increased risk of unintended consequences arising from mercury exposure.

### Mercury Excretion in Human Infants Who Became Autistic

Our findings are consistent with the hypothesis connecting mercury exposure and autism. Autistic infants released dramatically lower levels of mercury into hair than control infants. In our autistic group, this reduced level was not associated with lower levels of overall exposure, quite the contrary. In many, though not all, exposure categories, autistic infants experienced higher levels of mercury exposure. As a matter of design, we did not attempt to assess the impact of differences in vaccine exposures, because we only included controls who received a full exposure to mercury through the thimerosal in vaccines.

Autistic infants in our sample experienced increased exposure levels through maternal Rho D immunoglobulin injections (the large majority of licensed preparations sold during the study period used thimerosal as a preservative). Forty-three out of 94, or 46%, of the children in our sample were exposed to mercury through these injections, as compared to 4 out of 45, or 9%, of controls. Several of the autistic mothers received multiple injections, which resulted in a mean number of Rho D immunoglobulin injections in the autistic group of 0.52 injections per child, as compared to 0.09 among the controls. This observation is supported by a similar finding of elevated Rh incompatibility in mothers of autistic children in a previous study (Juul-Dam, Townsend, and Courchesne 2001). The level of Rh incompatibility we observed in our sample, however, is significantly greater than the rate observed in mothers in the previous study, 46% versus 12%. (The prevalence of Rh incompatibility in our controls was also higher, with a rate of 9% as compared to 3% in the previous study.) The rate of antenatal prophylaxis in our sample seems high and may be a result of increased treatment rates of Rh incompatibility as well as increasing frequency of the practice of antenatal prophylaxis, a relatively recent development. Because our study is the second report of elevated rates of autism in children born to Rh-negative mothers, this is a finding that deserves further investigation.

Increased numbers of amalgam fillings in the mother has been associated with increased fetal mercury exposure in several studies (Drasch et al. 1994; Vimy et al. 1997). Our results suggest that autistic children received increased exposure through outgassing of amalgam fillings than controls. The average level of amalgam fillings among mothers of autistic children was significantly greater than controls, with 8.35 fillings per mother in the autistic group ands 6.6 fillings among control mothers. Mothers of autistic children were far more likely to have received extensive dental work, with 35 of 94 mothers, or 37%, having 10 or more amalgam fillings as compared to 8 of 45, 18%, of controls.

Maternal dietary consumption of fish was not significantly associated with autism (data not shown).

Within the autistic group there were also strong differences in hair mercury levels. Lower hair mercury levels were significantly associated with the severity of the autistic behavior observed in the clinic. Adjusting for gender differences, these results were even stronger, because the "mildly autistic" group was disproportionately female. Within the mildly autistic group, female hair levels were almost uniformly lower than the male levels. This suggests that factors related to gender might offer a level of protection to female infants who might otherwise demonstrate more severe symptoms. By contrast, boys who displayed symptoms of similar severity nevertheless successfully released larger amounts of mercury, suggesting that boys might require high levels of mercury elimination to develop at similar rates. The increased male risk of autism has been extensively documented (Fombonne 1999; Gillberg and Wing 1999).

The control group showed a very strong correlation between measurable mercury exposure and the amount released into hair. This suggests that normal children have an ability to defend themselves against potentially toxic exposures and may demonstrate little negative effect despite exposures that were relatively large. By contrast, autistic infants who experienced comparable exposure to mercury were completely incapable of excreting mercury through hair at the levels that might have been predicted based on the excretion patterns of the control infants.

### Possible Consequences of Low Hair Mercury Levels

In past reviews of potential risk from mercury in vaccines (Stratton, Gable, and McCormick 2001), the possibility of neurological damage due to exposure to thimerosal has been minimized as a "theoretical, but unproven" risk. A core concern among reviewers has been the absence of evidence that "low-dose" exposures such as those administered through vaccines have the ability to cause any detectable harm.

Our study suggests two reasons why "low dose" (where "low" is relative to demonstrably harmful or even fatal doses and not the modeled EPA standard) exposures might raise the risk of developmental damage. First, vaccine exposures do not occur in isolation, but rather represent one among several pathways of exposure through which the fetal and infant brain might accumulate toxic levels of mercury. These pathways must therefore be evaluated in the context of cumulative exposures, any one of which might be harmless on its own but when combined with other sources might contribute to harmful overall levels. Both the autistic and the control children in our study showed increased mercury risk based on multiple sources of exposure: in the autistic group, both Rho D immunoglobulin and amalgam fillings in the mother were elevated relative to controls; in the control group, hair mercury levels were significantly correlated with maternal amalgam fillings and fish consumption as well as vaccine thimerosal exposure.

Second, the risk of any exposure will be greater if a larger fraction of the toxin is retained in tissue and not excreted quickly. Although hair is a minor pathway for mercury excretion and is far less important than feces and urine, the low levels of mercury in the hair of autistic infants support a hypothesis that these infants were retaining mercury in tissue at a higher rate than control infants. The lack of mercury in the hair of autistics may be due to a decrease in blood mercury levels feeding the hair follicles. This decrease is likely caused by the retention of the mercury inside the cells where it most likely causes its major biological damage.

When mercury is not available to the hair follicle, it is less likely to be available to the primary detoxification and excretory pathways and retained in tissue. If we presume that a portion of the tissue mercury retention is sequestered in the central nervous system and is available to cause neurological damage at sensitive points in brain development, then it is plausible that mercury-associated damage might be a meaningful element in the pathological process that leads to an outcome of autism.

### Limitations of the Current Study

We recognize that there are limitations to the current study. The study was not the result of a fully prospective design, recruitment of autistic study subjects was influenced by medical care-seeking behavior, the testing facilities were not under the direct control of the investigators, and the resultant population distributions may not be representative of the autism population as a whole. Additional research is necessary both to replicate these findings in autism and to elaborate on the impact of all the major risk factors associated with toxic exposures to mercury.

## CONCLUSIONS

The reduced levels of mercury in the first baby haircut of autistic infants raise clear questions about the detoxification capacity of a subset of infants. Despite hair levels suggesting low exposure, these infants had measured exposures at least equal to a control population, suggesting that control infants were able to eliminate mercury more effectively. In the case of autistic infants, those in our sample were exposed to higher levels of mercury during gestation, through dental amalgams or Rho D immunoglobulin injections in the mother. The addition of multiple postnatal exposures to mercury in childhood vaccines would have more severe consequences in infants whose detoxification capacity is reduced or who may be closer to a dangerous threshold exposure. In the case of control infants, mercury hair levels were strongly affected by exposure levels, suggesting that detoxification and excretion played an important role in ensuring normal development in children with elevate toxic exposure relative to peers. If reduced overall mercury elimination is related to hair elimination, then autistic infants will retain significantly higher levels of mercury in tissue, including the brain, than normal infants. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, our study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

## REFERENCES

Aronson, M., B. Hagberg, and C. Gillberg. 1997. Attention deficits and autism spectrum disorders in children exposed to alcohol during gestation: A follow-up study. *Dev. Med. Child Neurol.* 39:58–587.

Baird, G., T. Charman, S. Baron-Cohen, A. Cox, J. Swettenham, S. Wheelwright, and A. Drew. 2000. A screening instrument for autism at 18 months of age: A 6-year follow-up study. *J. Am. Acad. Child Adolesc. Psychiatry* 39:694–702.

Ballatori, N., and T. W. Clarkson. 1984. Dependence of biliary secretion of inorganic mercury on the biliary transport of glutathione. *Biochem. Pharmacol.* 33:1093–1098.

Bernard, S., A. Enayati, L. Redwood, H. Roger, and T. Binstock. 2001. Autism: A novel form of mercury poisoning. *Med. Hypotheses* 56:462–471.

Bertrand, J., A. Mars, C. Boyle, F. Bove, M. Yeargin-Allsopp, and P. Decoufle. 2001. Prevalence of Autism in a United States Population: The Brick Township, New Jersey, Investigation. *Pediatrics* 108:1155–1161.

Blaxill, M. F. 2001. Rising incidence of autism: Association with thimerosal. Presentation to Immunization Safety Review, Cambridge, MA. Available at: http://www.iom.edu/iom/iomhome.nsf/WFiles/Blaxill/$file/Blaxill.PDF

Bolton, P. F., R. J. Park, J. N. Higgins, P. D. Griffiths, and A. Pickles. 2002. Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. *Brain* 125(Pt 6):1247–1255.

Burd, L., W. Fisher, and J. Kerbeshian. 1987. A prevalence study of pervasive developmental disorders in North Dakota. *J. Am. Acad. Child Adolesc. Psychiatry* 26:700–703.

Centers for Disease Control and Prevention. 1999. Thimerosal in vaccines: A joint statement of the American Academy of Pediatrics and the Public Health Service. *MMWR Morb. Mortal. Wkly Rep.* 48:563–565.

Dally, A. 1997. The rise and fall of pink disease. *Soc. Hist. Med.* 10:291–304.

Deb, S., and K. B. Prasad. 1994. The prevalence of autistic disorder among children with a learning disability. *Br. J. Psychiatry* 165:395–399.

Department of Developmental Services. 1999. Changes in the population of persons with autism and pervasive developmental disorders in California's Developmental Services System: 1987–1998. A Report to the Legislature. Sacramento, CA. Available at: http://www.dds.ca.gov/autism/pdf/autism_report_1999.pdf

*Diagnostic and statistical manual of mental disorders*, 3rd edition (DSM-III). 1980, 87–90. Washington, DC: American Psychiatric Society.

*Diagnostic and statistical manual of mental disorders*, 3rd edition—revised (DSM-III-R). 1987, 38–39. Washington DC: American Psychiatric Society.

*Diagnostic and statistical manual of mental disorders*, 4th edition (DSM-IV). 1994, 66–71. Washington, DC: American Psychiatric Society.

Drasch, G., I. Schupp, H. Hofl, R. Reinke, and G. Roider. 1994. Mercury burden of human fetal and infant tissues. *Eur. J. Pediatr.* 153:607–610.

Eisenberg, L., and L. Kanner. 1956. Early Infantile Autism. *Am. J. Orthopsychiatr.* 26:556–566.

Fombonne, E. 1999. The epidemiology of autism: A review. *Psychol. Med.* 29:769–786.

Gentile, P. S., M. J. Trentalange, W. Zamichek, and M. Coleman. 1983. Brief report: Trace elements in the hair of autistic and control children. *J. Autism Dev. Disord.* 13:205–206.

Gillberg, C., and L. Wing. 1999. Autism: Not an extremely rare disorder. *Acta Psychiatr. Scand.* 99:399–406.

Grandjean, P., P. Weihe, R. F. White, and F. Debes. 1998. Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. *Environ. Res.* 77:165–172.

Grandjean, P., P. Weihe, R. F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N. Sorensen, R. Dahl, and P. J. Jorgensen. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.* 19:417–428.

Holloway, C., J. B. Adams, F. Castro, M. Kerr, and M. Margolis. 2001. Investigation of heavy metal toxicity in people with autism. Presentation to the International Meeting for Autism Research (IMFAR), San Diego, CA.

Hoshino, Y., H. Kumashiro, Y. Yashima, R. Tachibana, and M. Watanabe. 1982. The epidemiological study of autism in Fukushima-ken. *Folia Psychiatr. Neurol. Jpn.* 36:115–124.

Kales, S. N., and R. H. Goldman. 2002. Mercury exposure: Current concepts, controversies, and a clinic's experience. *J. Occup. Environ. Med.* 44:143–154.

Kaye, J. A., M. del Melero-Montes, and H. Jick. 2001. Mumps, measles, and rubella vaccine and the incidence of autism recorded by general practitioners: A time trend analysis. *BMJ* 322:460–463.

Juul-Dam, N., J. Townsend, and E. Courchesne. 2001. Prenatal, perinatal, and neonatal factors in autism, pervasive developmental disorder-not otherwise specified, and the general population. *Pediatrics* 107:E63.

Kostial, K., M. Blanusa, I. Rabar, and I. Simonovic. 1981. More data on mercury absorption in relation to dietary treatment in rats. *Toxicol. Lett.* 7:201–205.

Kostial, K., D. Kello, S. Jogo, I. Rabar, and T. Maljkovic. 1978. Influence of age on metal metabolism and toxicity. *Environ. Health Perspect.* 25:81–86.

Kostial, K., I. Rabar, M. Ciganovic, and I. Simonovic. 1979. Effect of milk on mercury absorption and gut retention in rats. *Bull. Environ. Contam. Toxicol.* 23:566–571.

Lotter, V. 1966. Epidemiology of autistic conditions in young children. I: Prevalence. *Soc. Psychiatry* 1:124–137.

Puchyr, R. F., D. A. Bass, R. Gajewski, M. Calvin, W. Marquardt, K. Urek, M. E. Druyan, and D. Quig. 1998. Preparation of hair for measurement of elements by inductively coupled plasma-mass spectrometry (ICP-MS). *Bio. Trace El. Res.* 62:167–182.

Ritvo, E. R., B. J. Freeman, C. Pingree, A. Mason-Brothers, L. Jorde, W. R. Jenson, W. M. McMahon, P. B. Petersen, A. Mo, and A. Ritvo. 1989. The UCLA-University of Utah epidemiologic survey of autism: Prevalence. *Am. J. Psychiatry* 146:194–199.

Rowland, I. R., M. J. Davies, and P. Grasso. 1978. Metabolism of methylmercuric chloride by the gastro-intestinal flora of the rat. *Xenobiotica* 8:37–43.

Rowland, I. R., R. D. Robinson, and R. A. Doherty. 1984. Effects of diet on mercury metabolism and excretion in mice given methylmercury: Role of gut flora. *Arch. Environ. Health* 39:401–408.

Rutter, M. 1978. Diagnosis and Definition. In *Autism: A reappraisal of concepts and treatments*, ed. M. Rutter and E. Schopler, 1–25. New York: Plenum Press.

Ryabukin, Y. S. 1978. Activation analysis of hair as an indicator of contamination of man by environmental trace element pollutants. International Atomic Energy Agency report, IAEA/RL/50, Vienna.

Salisbury, D. M., and S. Dittman. 1999. Immunization in Europe. In *Vaccines*, 3rd ed., ed. S. A. Plotkin and W. A. Orenstein, 1033–1046. New York: W.B. Saunders.

Seidel, S., R. Kreutzer, D. Smith, S. McNeel, and D. Gilliss. 2001. Assessment of commercial laboratories performing hair mineral analysis. *JAMA* 285:67–72.

Shearer, T. R., K. Larson, J. Neuschwander, and B. Gedney. 1982. Minerals in the hair and nutrient intake of autistic children. *J. Autism Dev. Disord.* 12:25–34.

Steffenburg, S., C. L. Gillberg, U. Steffenburg, and M. Kyllerman. 1996. Autism in Angelman syndrome: A population-based study. *Pediatr. Neurol.* 14:131–136.

Stratton, K., A. Gable, and M. McCormick, eds. 2001. *Immunization safety review: Thimerosal containing vaccines and neurodevelopmental disorders.* Institute of Medicine, Washington, DC: National Academy Press.

Stromland, K., V. Nordin, M. Miller, B. Akerstrom, and C. Gillberg. 1994. Autism in thalidomide embryopathy: A population study. *Dev. Med. Child Neurol.* 36:351–356.

Summers, A. O., J. Wireman, M. J. Vimy, F. L. Lorscheider, B. Marshall, S. B. Levy, S. Bennett, and L. Billard. 1993. Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. *Antimicrob. Agents Chemother.* 37:825–834.

Taylor, B., E. Miller, C. P. Farrington, M. C. Petropoulos, I. Favot-Mayaud, J. Li, and P. A. Waight. 1999. Autism and measles, mumps, and rubella vaccine: No epidemiological evidence for a causal association. *Lancet* 353: 2026–2029.

Treffert, D. A. 1970. Epidemiology of infantile autism. *Arch. Gen. Psychiatry* 22:431–438.

Vimy, M. J., D. E. Hooper, W. W. King, and F. L. Lorscheider. 1997. Mercury from maternal "silver" tooth fillings in sheep and human breast milk. A source of neonatal exposure. *Biol. Trace Elem. Res.* 56:143–152.

Wahlstrom, J., C. Gillberg, K. H. Gustavson, and G. Holmgren. 1986. Infantile autism and the fragile X. A Swedish multicenter study. *Am. J. Med. Genet.* 23:403–408.

Wecker, L., S. B. Miller, S. R. Cochran, D. L. Dugger, and W. D. Johnson. 1985. Trace element concentrations in hair from autistic children. *J. Ment. Defic. Res.* 29(Pt 1):15–22.

Williams, P. G., and J. H. Hersh. 1997. A male with fetal valproate syndrome and autism. *Dev. Med. Child Neurol.* 39:632–634.

Wing, L., and J. Gould. 1979. Severe impairments of social interaction and associated abnormalities in children: Epidemiology and classification. *J. Autism Dev. Disord.* 9:11–29.

6

# Association Between Thimerosal-Containing Vaccine and Autism

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**Context** Mercuric compounds are nephrotoxic and neurotoxic at high doses. Thimerosal, a preservative used widely in vaccine formulations, contains ethylmercury. Thus it has been suggested that childhood vaccination with thimerosal-containing vaccine could be causally related to neurodevelopmental disorders such as autism.

**Objective** To determine whether vaccination with a thimerosal-containing vaccine is associated with development of autism.

**Design, Setting, and Participants** Population-based cohort study of all children born in Denmark from January 1, 1990, until December 31, 1996 (N=467450) comparing children vaccinated with a thimerosal-containing vaccine with children vaccinated with a thimerosal-free formulation of the same vaccine.

**Main Outcome Measures** Rate ratio (RR) for autism and other autistic-spectrum disorders, including trend with dose of ethylmercury.

**Results** During 2986654 person-years, we identified 440 autism cases and 787 cases of other autistic-spectrum disorders. The risk of autism and other autistic-spectrum disorders did not differ significantly between children vaccinated with thimerosal-containing vaccine and children vaccinated with thimerosal-free vaccine (RR, 0.85 [95% confidence interval {CI}, 0.60-1.20] for autism; RR, 1.12 [95% CI, 0.88-1.43] for other autistic-spectrum disorders). Furthermore, we found no evidence of a dose-response association (increase in RR per 25 μg of ethylmercury, 0.98 [95% CI, 0.90-1.06] for autism and 1.03 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

**Conclusion** The results do not support a causal relationship between childhood vaccination with thimerosal-containing vaccines and development of autistic-spectrum disorders.

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HIGH DOSES OF MERCURIC COMpounds are nephrotoxic and neurotoxic.[1] Thimerosal, an organic compound that contains ethylmercury, has been widely used since the 1930s as a preservative in certain vaccines. In the 1990s, an increasing number of different vaccines containing thimerosal were introduced in immunization schedules around the world, and thus the average cumulative exposure to thimerosal in infants has increased in recent years. This has led to the suggestion that childhood vaccination with thimerosal-containing vaccines increases the risk of neurodevelopmental disorders, such as autism, attention-deficit/hyperactivity disorder, and language and speech delay.

In a recent independent review conducted by the Immunization Safety Committee, on behalf of the Institute of Medicine, it was concluded that the evidence was inadequate to accept or reject a causal relationship between thimerosal-containing vaccine and neurodevelopmental disorders.[2] However, based on comparison with the toxicology of methylmercury, the biological plausibility of a link remained. Further research was recommended. We examined the hypothesized association by comparing children vaccinated with a thimerosal-containing pertussis vaccine with children vaccinated with the same pertussis vaccine formulated without thimerosal and following them with respect to development of autism and other autistic-spectrum disorders.

## METHODS

The Danish childhood vaccination program is voluntary and free of charge to the vaccinees. Vaccines against diphtheria, tetanus, polio, measles, mumps, rubella, pertussis, and *Haemophilus influenzae* type b are administered by general practitioners.[3] From 1970, the only thimerosal-containing vaccine in the program has been the whole-cell pertussis vaccine. In late March 1992, the last batch of thimerosal-containing whole-cell pertussis vaccine was released and distributed from Statens Serum Institut. Only the whole-cell vaccine produced by Statens Serum Institut has been used in Denmark. The same vaccine was reformulated without thimerosal and used until January 1, 1997, when it was replaced with an acellular pertussis vaccine.[4] The whole-cell vaccine was administered at 5 weeks, 9 weeks, and 10 months from 1970 and until it was replaced, irrespective of thimerosal content.[3] The thimerosal formulation contained 50 μg of thimerosal (~25 μg of ethylmercury) in the first

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dose and 100 µg (~50 µg of ethylmercury) in each of the succeeding 2 doses.

Since April 1968, all persons in Denmark have been given a unique identification number in the Danish Civil Registration System.[5] Based on this registry, we constructed a cohort consisting of all children born in Denmark in the period from January 1, 1990, to December 31, 1996. Using the unique personal identification number, we were able to link information on vaccinations, diagnoses of autism, diagnoses of other autistic-spectrum disorders, other relevant diagnoses, and potential confounders to the children in the cohort. The dates of vaccination with 1, 2, or 3 doses of whole-cell pertussis vaccine were obtained from the National Board of Health. We have published details of this process in a study of autistic-spectrum disorders and measles-mumps-rubella vaccine.[6] Doses administered before June 1, 1992, were considered to contain thimerosal, and doses administered after June 1, 1992, were considered thimerosal-free. Children who received thimerosal-free vaccine after 1 or 2 doses of thimerosal-containing vaccine were classified only according to receipt of thimerosal-containing vaccine.

Information on autism and other autistic-spectrum disorder diagnoses was obtained from the Danish Psychiatric Central Register.[6,7] Child psychiatrists make the diagnosis and assign diagnostic codes for this register. In the period 1991-1993, the *International Classification of Diseases, 8th Revision* (*ICD-8*) was used. In the period 1994 through 2000, the *International Classification of Diseases, 10th Revision* (*ICD-10*) was used. All cases of autism and other autistic-spectrum disorders in our study have been ascertained using *ICD-10*. Autism was defined by *ICD-10* code F84.0, which is similar to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*) code 299.00, and other autistic-spectrum disorders were defined by *ICD-10* codes F84.1-F84.9, which are similar to *DSM-IV* codes 299.10 and 299.80.

In 1991-1994, only inpatients were included in the Danish Psychiatric Central Register. From 1995, both inpatients and outpatients were included. Information on diagnoses of tuberous sclerosis, Angelman syndrome, fragile X syndrome, and congenital rubella, conditions associated with autism, was obtained from the National Hospital Discharge Register.[8] Information on possible confounding factors was obtained from the Danish Civil Registration System and the Danish Medical Birth Registry,[9] as follows: child's sex, child's place of birth (Copenhagen, Copenhagen suburbs, area with ≥100 000 population, area with population of 10 000-99 999, area with population of <10 000), birth weight (<2500, 2500-2999, 3000-3499, 3500-3999, ≥4000 g), 5-minute Apgar score (0-7, 8-9, 10), gestational age (<37, 37-41, ≥42 weeks), mother's age at birth of child (<20, 20-24, 25-29, 30-34, 35-39, and ≥40 years), and mother's country of birth (Danish or not). The percentage of missing values for the variables birth weight, gestational age, 5-minute Apgar score, mother's country of birth, and child's place of birth were 6.6%, 6.9%, 6.9%, 0.3%, and 0.03%, respectively.

Children in our cohort contributed person-time to follow-up from 1 year of age or January 1, 1991, whichever occurred last, until a diagnosis of autism, other autistic-spectrum disorder, tuberous sclerosis, Angelman syndrome, fragile X syndrome or congenital rubella, possible death, disappearance or emigration, 11 years of age, or until December 31, 2000, whichever occurred first. Follow-up was begun at 1 year of age because indications for an evaluation of a possible case of autistic-spectrum disorder typically occur after the first year of life. The resulting incidence rates for autism and other autistic-spectrum disorders were analyzed with Poisson regression, producing estimates of rate ratios (RRs) according to vaccination history.[10] Vaccination history was considered a time-varying variable. We estimated the dose-response relationship between thimerosal-containing vaccine and autism and other autistic-spectrum disorders as the increase in RR per 25 µg of ethylmercury. We adjusted all RRs for age (1-9 years of age, ½-year intervals; 10 years of age, 1-year interval) and calendar period (1991-1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000). We further adjusted our estimates for the potential confounding variables previously listed. Statistical analysis was performed using PROC GENMOD in SAS version 6.12 (SAS Institute Inc, Cary, NC).

## RESULTS

A total of 467 450 children were born in Denmark between January 1, 1990, and December 31, 1996. During 2 986 654 person-years of follow-up, we identified 440 cases of autism and 787 cases of other autistic-spectrum disorders. The mean (SD) age at diagnosis was 4.7 (1.7) years for autism and 6.0 (1.9) years for other autistic-spectrum disorders. The follow-up of 5770 children was prematurely terminated because of death (n=579), emigration (n=5035), disappearance (n=87), tuberous sclerosis (n=51), Angelman syndrome (n=17), or congenital rubella (n=1).

In our cohort, only 20 755 (4.4%) children did not receive any whole-cell pertussis vaccine, 446 695 (95.6%) were vaccinated at least once, 416 081 (89.0%) were vaccinated twice, and 293 186 (62.7%) received 3 doses of whole-cell pertussis vaccine. Among those who received at least 1 thimerosal-containing pertussis vaccine (n=138 953), 118 593 received 1 subsequent dose and 65 725 received 2 subsequent doses of thimerosal-containing vaccine. Furthermore, 42 032 children who received at least 1 dose of thimerosal-containing vaccine subsequently received at least 1 dose of thimerosal-free vaccine. In those receiving at least 1 dose of whole-cell pertussis vaccine, there were 407 cases of autism (303 receiving thimerosal-free and 104 receiving thimerosal-containing vaccine) and 751 cases of other autistic-spectrum disorders (430 receiving thimerosal-free and 321 receiving thimerosal-containing vaccine).

Comparing children vaccinated with at least 1 dose of thimerosal-containing whole-cell pertussis vaccine with children vaccinated with a thimerosal-free formulation of the same vaccine, we found a fully adjusted RR of 0.85 (95% confidence interval [CI], 0.60-1.20) for autism and an RR of 1.12 (95% CI, 0.88-1.43) for other autistic-spectrum disorders (TABLE). Furthermore, we found no evidence of a dose-response association between the dose of ethylmercury received and autistic-spectrum disorders (increase in RR per 25 µg of ethylmercury, 0.98 [95% CI, 0.90-1.06] for autism and 1.03 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

Although doses administered after June 1, 1992, were considered thimerosal-free, it is conceivable that a few thimerosal-containing doses may have been administered during the months after this date. To assess whether misclassification of vaccine type might have biased our estimates, we reestimated the RRs, omitting children vaccinated from June 1, 1992, through December 31, 1992. We found a fully adjusted RR of 0.87 (95% CI, 0.61-1.23) for autism and an RR of 1.15 (95% CI, 0.90-1.47) for other autistic-spectrum disorders and no evidence of a dose-response association (increase in RR per 25 µg of ethylmercury, 0.98 [95% CI, 0.90-1.07] for autism and 1.04 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

In a further analysis we evaluated the robustness of our results by restricting our cohort to children born in 1991-1993, a presumably more homogeneous group with respect to diagnosis, length of follow-up, and factors not included in this study (eg, mercury exposure through food) and found a fully adjusted RR of 0.86 (95% CI, 0.53-1.39) for autism and an RR of 1.05 (95% CI, 0.77-1.44) for other autistic-spectrum disorders and no evidence of a dose-response association (increase in RR per 25 µg of ethylmercury, 0.97 [95% CI, 0.85-1.10] for autism and 1.04 [95% CI, 0.96-1.13] for other autistic-spectrum disorders).

Finally, we evaluated the impact of missing values by the method of single imputation, replacing a missing value with the most common value of the relevant variable, and found a fully adjusted RR of 0.85 (95% CI, 0.60-1.20) for autism and 1.13 (95% CI, 0.89-1.44) for other autistic-spectrum disorders.

To evaluate whether the incidence of autistic-spectrum disorders was increasing in Denmark in the study period, we calculated time period trends from our cohort. We found statistically significant increases in age-adjusted RR per calendar year for both autism and other autistic-spectrum disorders during the study period (RR, 1.24 [95% CI, 1.17-1.31] for autism; RR, 1.21 [95% CI, 1.16-1.27] for other autistic-spectrum disorders). In the period from January 1, 1995, to December 31, 2000, a period where outpatients were included, we found similar trends (RR, 1.24 [95% CI, 1.16-1.32] for autism; RR, 1.20 [95% CI, 1.13-1.26] for other autistic-spectrum disorders).

## COMMENT

We found no evidence of an association between thimerosal-containing vaccine and autism in children who received thimerosal-containing vaccine compared with children who received the same vaccine formulated without thimerosal. Furthermore, there was no indication of a dose-response association between autism and the amount of ethylmercury received through thimerosal.

The hypothesis of an association between thimerosal and autism has primarily been based on biological plausibility through analogies with methylmercury.[2] Ethylmercury, however, is thought to have a shorter half-life in the human body than methylmercury, and no controlled studies of low-dose ethylmercury toxicity in humans have been conducted.[11] Pichichero and colleagues[12] measured the concentration of mercury in the blood, urine, and stool of infants who received thimerosal-containing vaccines and concluded that vaccination did not raise the blood concentration of mercury above safe limits, and that ethylmercury was rapidly

**Table.** Rate Ratio of Autism and Other Autistic-Spectrum Disorders Comparing Children Vaccinated With a Thimerosal-Containing Vaccine to Children Vaccinated With a Thimerosal-Free Formulation of the Same Vaccine

| | | Autism | | | Other Autistic-Spectrum Disorders | | |
|---|---|---|---|---|---|---|---|
| | Person-Years at Risk | No. of Cases | RR (95% CI)* | RR (95% CI)† | No. of Cases | RR (95% CI)* | RR (95% CI)† |
| Vaccinations | | | | | | | |
| All thimerosal-free | 1 660 159 | 303 | 1.00 | 1.00 | 430 | 1.00 | 1.00 |
| Any containing thimerosal | 1 220 006 | 104 | 0.85 (0.60-1.20) | 0.85 (0.60-1.20) | 321 | 1.12 (0.88-1.43) | 1.12 (0.88-1.43) |
| Doses of thimerosal-containing vaccine | | | | | | | |
| None | 1 660 159 | 303 | 1.00 | 1.00 | 430 | 1.00 | 1.00 |
| 1 dose (25 µg eHg) | 169 920 | 18 | 0.99 (0.59-1.68) | 1.01 (0.60-1.71) | 40 | 0.96 (0.67-1.39) | 0.95 (0.66-1.37) |
| 2 doses (75 µg eHg) | 447 973 | 33 | 0.71 (0.46-1.09) | 0.70 (0.46-1.09) | 130 | 1.20 (0.92-1.56) | 1.20 (0.92-1.56) |
| 3 doses (125 µg eHg) | 602 113 | 53 | 0.96 (0.63-1.46) | 0.96 (0.63-1.47) | 151 | 1.11 (0.83-1.48) | 1.13 (0.84-1.51) |
| Trend (increase in RR per 25 µg eHg) | | | 0.98 (0.90-1.06) | 0.98 (0.90-1.06) | | 1.03 (0.97-1.09) | 1.03 (0.98-1.09) |

Abbreviations: CI, confidence interval; eHg, ethylmercury; RR, rate ratio.
*Adjusted for confounders: age and calendar period.
†Fully adjusted: age, calendar period, child's sex, child's place of birth, birth weight, 5-minute Apgar score, gestational age, mother's age at birth of child, and mother's country of birth.

eliminated via the stools. They estimated the blood half-life of ethylmercury at 7 days (95% CI, 4-10 days), although their study was not designed as a formal pharmacokinetic study of ethylmercury.

In 1999, when thimerosal was still widely used, children in the US childhood immunization program would have received 187.5 µg of ethylmercury by the age of 6 months and 237.5 µg of ethylmercury by the age of 2 years.[2] In Denmark, children would have received 125 µg of ethylmercury by the age of 10 months. However, in the Danish program, children received larger doses of ethylmercury per vaccine (50 µg compared with 25 µg in the United States) so that at 3 months, Danish children would have received the same amount of ethylmercury as US children (75 µg).[2]

To our knowledge, our study is the first population-based cohort study to examine the association between thimerosal and autism. In Denmark since 1970, only the whole-cell pertussis vaccine was formulated with thimerosal, and this vaccine was the only one used for pertussis immunization until it was replaced with an acellular pertussis vaccine in 1997. The unique situation has allowed a direct comparison of children vaccinated with a thimerosal-containing whole-cell pertussis vaccine with children vaccinated with the same vaccine formulated without thimerosal, and thus we have avoided confounding by contraindication and other selection bias associated with unvaccinated children. Furthermore, we have no reason to believe that the 2 groups of children differ with respect to other potential risk factors for autism.

All data used in this study were collected prospectively, eliminating concerns about recall bias. Madsen and colleagues[6] reviewed the medical records of 40 children with autism from the Danish Psychiatric Central Register and found that 37 children met the operational criteria for autism according to a systematic coding scheme developed by the Centers for Disease Control and Prevention.[13] Furthermore, Madsen and colleagues[6] found Danish prevalence rates for autism and other autistic-spectrum disorders comparable to prevalence rates found in other studies. Thus we conclude that the validity and completeness of the autism and other autistic-spectrum disorder diagnoses in the Danish Psychiatric Central Register is high. However, it is possible that the National Hospital Discharge Register is not complete with respect to a diagnosis of tuberous sclerosis, Angelman syndrome, fragile X syndrome, and congenital rubella. However, these conditions are rare in the general population and since we have compared only vaccinated children, lack of completeness is unlikely to seriously confound an association between thimerosal content and autistic-spectrum disorder.

We found statistically significant increased rates over time for both autism and other autistic-spectrum disorders. These results are compatible with a dramatic increase in the number of diagnosed cases of autistic-spectrum disorders during the study period, similar to what has been observed in other countries (eg, the United States).

In Denmark, general practitioners administer all childhood vaccinations and are reimbursed when reporting these to the National Board of Health, thus ensuring a high degree of completeness. In our cohort we found that 96%, 89%, and 63% of children were vaccinated at least once, at least twice, and 3 times with whole-cell pertussis vaccine. The low uptake of 3 doses is unexpected but can be partially explained by the transition to acellular pertussis vaccine in January 1997. Furthermore, for each dose there is a small chance of either missing the dose or the vaccination not being recorded. Even small probabilities for each dose can, if they are statistically independent, result in a significant reduction in the calculated uptake of all 3 doses.

A possible weakness of this study is that the date of diagnosis used as the incidence date may differ significantly from the "onset of symptoms" date. A diagnosis of autistic-spectrum disorder can be a lengthy process; this is reflected in the mean ages of diagnoses in this study (4.7 years for autism and 6.0 years for other autistic-spectrum disorders). However, this is more likely to be a problem in an incidence study than in a risk factor study.

In conclusion, our results are not compatible with the hypothesis of a causal association between thimerosal and autistic-spectrum disorders.

**Author Contributions:** *Study concept and design:* Hviid, Stellfeld, Wohlfahrt, Melbye.
*Analysis and interpretation of data:* Hviid, Wohlfahrt.
*Drafting of the manuscript:* Hviid.
*Critical revision of the manuscript for important intellectual content:* Stellfeld, Wohlfahrt, Melbye.
*Statistical expertise:* Hviid, Wohlfahrt.
*Obtained funding:* Hviid, Melbye.
*Study supervision:* Wohlfahrt, Melbye.

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REFERENCES

1. Clarkson TW. The toxicology of mercury. *Crit Rev Clin Lab Sci.* 1997;34:369-403.
2. Stratton K, Gable A, McCormick MC, eds. *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders.* Washington, DC: National Academy Press; 2001.
3. Plesner AM, Ronne T. The childhood vaccination program: background, status and future [in Danish]. *Ugeskr Laeger.* 1994;156:7497-7503.
4. Ronne T. The Danish vaccination program for children [in Danish]. *Ugeskr Laeger.* 1997;159:1584-1585.
5. Malig C. *The Civil Registration System in Denmark.* Bethesda, Md: International Institute for Vital Registration and Statistics; 1996. IIVRS Technical Paper No. 6.
6. Madsen KM, Hviid A, Vestergaard M, et al. A population-based study of measles, mumps, and rubella vaccination and autism. *N Engl J Med.* 2002;347:1477-1482.
7. Munk-Jorgensen P, Mortensen PB. The Danish Psychiatric Central Register. *Dan Med Bull.* 1997;44:82-84.
8. Andersen TF, Madsen M, Jorgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register: a valuable source of data for modern health sciences. *Dan Med Bull.* 1999;46:263-268.
9. Knudsen LB, Olsen J. The Danish Medical Birth Registry. *Dan Med Bull.* 1998;45:320-323.
10. Clayton D, Hills M. *Statistical Models in Epidemiology.* Oxford, England: Oxford University Press; 1993.
11. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics.* 2001;107:1147-1154.
12. Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: a descriptive study. *Lancet.* 2002;360:1737-1741.
13. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. *Pediatrics.* 2001;108:1155-1161.


 

7

# Thimerosal and the Occurrence of Autism: Negative Ecological Evidence From Danish Population-Based Data

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ABSTRACT. *Objective.* It has been suggested that thimerosal, a mercury-containing preservative in vaccines, is a risk factor for the development of autism. We examined whether discontinuing the use of thimerosal-containing vaccines in Denmark led to a decrease in the incidence of autism.

*Design.* Analysis of data from the Danish Psychiatric Central Research Register recording all psychiatric admissions since 1971, and all outpatient contacts in psychiatric departments in Denmark since 1995.

*Patients.* All children between 2 and 10 years old who were diagnosed with autism during the period from 1971–2000.

*Outcome Measures.* Annual and age-specific incidence for first day of first recorded admission with a diagnosis of autism in children between 2 and 10 years old.

*Results.* A total of 956 children with a male-to-female ratio of 3.5:1 had been diagnosed with autism during the period from 1971–2000. There was no trend toward an increase in the incidence of autism during that period when thimerosal was used in Denmark, up through 1990. From 1991 until 2000 the incidence increased and continued to rise after the removal of thimerosal from vaccines, including increases among children born after the discontinuation of thimerosal.

*Conclusions.* The discontinuation of thimerosal-containing vaccines in Denmark in 1992 was followed by an increase in the incidence of autism. Our ecological data do not support a correlation between thimerosal-containing vaccines and the incidence of autism. *Pediatrics* 2003; 112:604–606; *autism, vaccine, thimerosal, mercury, population, epidemiology.*

ABBREVIATIONS. *ICD-8, International Classification of Diseases, Eighth Revision; ICD-10, International Classification of Diseases, 10th Revision.*

There has been concern that there may be an association between thimerosal, a vaccine preservative that contains ethyl mercury, and neurodevelopmental outcomes, including autism.[1,2] Findings in the field of methyl mercury have been used to suggest causality. Prenatal exposure to low doses of methyl mercury has been associated with subtle neurodevelopmental abnormalities in some studies,[3,4] and symptoms of autism and methyl mercury intoxication have been claimed to be similar.[1] More research has been requested,[5] and a recent study of the concentrations of mercury after exposure to thimerosal-containing vaccines concluded that thimerosal poses very little risk to full-term infants.[6] In Denmark, thimerosal was used in childhood vaccines from the early 1950s until 1992. The objective of our study was to assess the incidence rates of autism among children between 2 and 10 years old before and after removal of thimerosal from vaccines to see if the discontinuation led to a decrease in the incidence of autism.

## PARTICIPANTS AND METHODS

For this study, the period of use of thimerosal vaccines was limited to 1961 until its discontinuation in March 1992 because information about the diagnosis of autism has only been obtainable from a nationwide computerized registration system, the Danish Psychiatric Central Research Register,[7] since 1969 and only children born in 1961 or later were at risk of developing autism before 10 years of age. Thimerosal was used during 1961–1970 in the diphtheria-tetanus-pertussis vaccines given in 4 doses when the child was 5, 6, 7, and 15 months old, and during 1970–1992 in the whole-cell pertussis vaccines given in 3 doses when the child was 5 weeks, 9 weeks, and 10 months old. The concentrations used in the vaccines from 1961–1970 and from 1970–1992 were 0.01% = 0.1 mg thimerosal, which equals 50 μg ethyl mercury per mL vaccine. The amount of vaccine given was 1 mL, except for the first dose of monocomponent pertussis vaccine where it was only 0.5 mL from 1970–1992. This means that children who followed the full vaccination program during the period 1961–1970 had received a total of 400 μg of thimerosal or 200 μg of ethyl mercury by the age of 15 months and during the period 1970–1992 they had received a total of 250 μg of thimerosal or 125 μg of ethyl mercury at 10 months of age. In March 1992 the last batch of thimerosal-containing vaccine was released and distributed from Statens Serum Institut in Denmark. All vaccinations were given free of charge and acceptance of vaccinations in Denmark has always been very high; from 1979 onward data on vaccination coverage was available and coverage rates of >90% were found (information was obtained from the State Serum Institute). Whether the toxicity of methyl mercury and ethyl mercury is the same remains controversial[8,9] but the recommended safe intake level of methyl mercury is estimated to be 0.1 μg/kg body weight/day by the US Environmental Protection Agency.[10] However, other federal regulatory agencies have recommended slightly higher levels.[9]

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PEDIATRICS (ISSN 0031 4005). 

Psychiatric inpatient treatment in Denmark has been reported to the Danish Psychiatric Central Research Register since 1969, and since 1995 outpatient activities were registered as well, providing the opportunity to examine long-term trends of the occurrence of autism in a total national population. In Denmark, inpatients refer both to children who stay at the hospital overnight and to children who come to the hospital on a daily basis for evaluation and treatment. The proportion of outpatient to inpatient activities was about 4 to 6 times as many outpatients as inpatients with variations across time and age bands. We obtained information on all children who from the second birthday up to, but not including the 10th birthday were diagnosed with autism in the period from January 1, 1971 to December 31, 2000 in the Danish Psychiatric Central Research Register during which period the register is assumed to be complete. The diagnosis of autism in children <2 years of age was considered uncertain. All individuals in Denmark are assigned a unique personal identification number[11] which is used in all national registers. Admissions to psychiatric hospitals in Denmark are coded using this CPR-number, which eliminates the risk of double-counting of cases. The date of onset was defined as the first day of the first admission leading to a diagnosis of psychosis proto-infantilis (*International Classification of Diseases, Eighth Revision* [*ICD-8*]: 299.00) or psychosis infantilis posterior (*ICD-8*: 299.01) or from 1994 onward, infantile autism (*International Classification of Diseases, 10th Revision* [*ICD-10*]: F84.0) or atypical autism (*ICD-10*: F84.1).[12,13]

### Statistics

Incidence rates were calculated for each year 1971–2000 using the age and gender specific number of persons in Denmark as a denominator. For each year and age band, we calculated the incidence as the number of people who at that age band and year was diagnosed with autism for the first time divided by the total number of people alive and living in Denmark at that age band and year.

## RESULTS

A total of 956 children with a male to female ratio of 3.5:1 had been diagnosed with autism during the period 1971–2000. Figure 1 shows the incidence rates according to calendar year and age band. The incidence was stable until 1990 and thereafter it increased in all age groups until 1999. Generally, rates were lower in 2000 than in 1999. Further subdivision by gender had no impact on these results (data not shown). In additional analyses we examined data using inpatients only. This was done to elucidate the contribution of the outpatient registration to the change in incidence. The same trend with an increase in the incidence rates from 1990 until the end of the study period was seen (data not shown).

There was no trend toward an increase in the incidence of autism during the period when thimerosal was used up to 1990. The incidence of autism began to increase in 1991, but continued to rise after the discontinuation of thimerosal (Fig 1), including increases among children born after 1992 (ie, the peak autism incidence in 1999 among children aged 2 to 4 and 5 to 6 years of age corresponds to children born in 1993–1997 after the introduction of thimerosal-free vaccines).

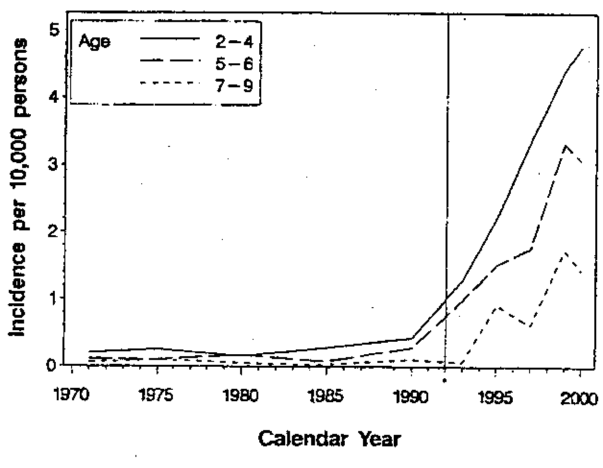


Fig 1. Incidence of autism by age and calendar year. The asterisk (*) indicates removal of thimerosal-containing vaccines in 1992.

## DISCUSSION

This study investigated if the discontinuation of thimerosal-containing vaccines paralleled a decrease in the occurrence of autism. The incidence of autism remained fairly constant during the period of use of thimerosal in Denmark, and the rise in incidence beginning in 1991 continued even in the group of children born after the discontinuation of thimerosal. The amount of thimerosal used in vaccines changed during the study period with less amount of thimerosal administered in the period 1970–1992. Moreover, the thimerosal-containing vaccine was gradually phased out meaning that the incidence rates should decline gradually if thimerosal has any impact on the development of autism. However, an increase (rather than a decrease) in the incidence rates of autism was observed.

Only very few incidence studies of autism have been made, and we found similar incidence rates and the same trend of increasing rates of autism in our study compared with studies conducted in other countries.[14,15] The increase in the incidence of autism from 1990 on may be attributable to more attention being drawn to the syndrome of autism and to a change in the diagnostic criteria from the *ICD-8* to the *ICD-10* in 1994. Also, outpatient activities were included in the Danish Psychiatric Central Research Register in 1995 and because many patients with autism in former years have been treated as outpatients this may exaggerate the incidence rates, simply because a number of patients attending the child psychiatric treatment system before 1995 were recorded for the first time, and thereby counted as new cases in the incidence rates.

## CONCLUSIONS

The discontinuation of thimerosal-containing vaccines in Denmark in 1992 was followed by an increase in the incidence of autism. Our ecological data do not support correlation between thimerosal-containing vaccines and the incidence of autism. Our data cannot, of course, exclude the possibility that thimerosal at doses larger than used in Denmark may lead to neurodevelopmental damage.


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## REFERENCES

1. Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. *Med Hypotheses*. 2001;56:462–471
2. Nelson KB, Bauman ML. Thimerosal and autism [commentary]? *Pediatrics*. 2003;111:674–679
3. Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Al Tikriti S. Fetal methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. *Arch Neurol*. 1987;44:1017–1022
4. Cox C, Clarkson TW, Marsh DO, Amin-Zaki L, Tikriti S, Myers GG. Dose-response analysis of infants prenatally exposed to methyl mercury: an application of a single compartment model to single-strand hair analysis. *Environ Res*. 1989;49:318–332
5. Institute of Medicine. *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders*. Washington, DC: National Academy Press; 2001
6. Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. *Lancet*. 2002;360:1737–1741
7. Munk-Jørgensen P, Mortensen PB. The Danish Psychiatric Central Register. *Dan Med Bull*. 1997;44:82–84
8. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines [review article]. *Pediatrics*. 2001;107:1147–1154
9. Clarkson TW. The three modern faces of mercury. *Environ Health Perspect*. 2002;110(suppl 1):11–23
10. Environmental Protection Agency. *Mercury Study Report to Congress: Volume 1, Executive Summary*. Washington, DA: Environmental Protection Agency; 1997. Publ. No. EPA 452/R-97-003
11. Malig C. *The Civil Registration System in Denmark*. IIVRS Technical Paper No. 66. Bethesda, MD: International Institute for Vital Registration and Statistics; 1996
12. World Health Organization. *Manual of the International Classification of Diseases, Eighth Revision (ICD-8)*. Geneva, Switzerland: World Health Organization; 1967
13. World Health Organization. *The International Classification of Diseases, 10th Revision (ICD-10) Classification of Mental and Behavioral Disorders: Diagnostic Criteria for Research*. Geneva, Switzerland: World Health Organization; 1993
14. Powell JE, Edwards A, Edwards M, Pandit BS, Sungum-Paliwal SR, Whitehouse W. Changes in the incidence of childhood autism and other autistic spectrum disorders in preschool children from two areas of the West Midlands, UK. *Dev Med Child Neurol*. 2000;42:624–628
15. Kaye JA, Mar Melero-Montes M, Jick H. Mumps, measles, and rubella vaccine and the incidence of autism recorded by general practitioners: a time trend analysis. *BMJ*. 2001;322:460–463

## FAT BABIES AND HEALTH

"In a recent issue an English contemporary calls attention to the mischief that is being done by the present standard that is accepted as regards healthy babies. As this paper well says, at baby shows the prize is practically always given to the fattest baby. There is a tradition current among mothers, as far as the memory of man runneth, that fat babies are just the pink of perfection. The surest index of this is that all manufacturers of artificial infant food advertise their wondrous virtues by photographs of thoroughly rounded, and at times positively obese dumplings of babies. Mothers are very proud of their young hopefuls if they are a mass of curves and dimples with deep folds at all the joints and cushions of fat that conceal their anatomy so effectively as to make them formless little masses of humanity."

*JAMA 100 years ago. JAMA*. 2003;289:1866

*Editor's Note*: Not much change in 100 years! Will we ever win this one?

Noted by JFL, MD

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# Neurotoxic Character of Thimerosal and the Allometric Extrapolation of Adult Clearance Half-time to Infants†

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Key words: methylmercury; ethylmercury; thimerosal; vaccination; toxicity; infants; kinetics; allometric extrapolation.

**The decomposition rate of organomercurials and the potency of the blood–brain barrier increase with the size of the organic radical. Thus methylmercury damages the brain more than thimerosal does, and when intake limits set for methylmercury are applied to thimerosal the safety margin is increased even if the clearances were the same. However, the clearance half-time of ethylmercury in adults is about one-third of the 50 days' clearance half-time of methylmercury given for 60 kg body weight. Moreover, because metabolic rates (e.g. basal metabolism, daily loss of mercury in per cent of body burden) in different weight groups are related to the fractional power of body weight (rule of allometry), mercury clears from the infant body faster than from the adult body. Blood mercury concentrations observed after vaccination showed agreement with allometrically extrapolated concentrations.** 

## INTRODUCTION

The germicidal effect of mercury in its different forms has entered a new phase in the past century when, as a preservative, sodium ethylmercury thiosalicylate (proprietary names: thimerosal, thiomersal, methiolate sodium) was added to commercial products of human plasma, immunoglobulins (immunoglobulin G; hepatitis B immune globulin) and vaccines (adsorbed diphtheria, tetanus and pertussis, hepatitis B, *haemophylus influenzae* and influenza). The next milestone in the history of ethylmercury thiosalicylate was the joint statement on thimerosal in vaccines by the American Academy of Pediatrics and the Public Health Service (1999). The statement was not alarmist but opened discussion and debate (Pless and Risber, 2000; Ball *et al.*, 2001; Freed *et al.*, 2002) on the use of ethylmercury thiosalicylate in vaccines.

## COMPARATIVE TOXICOLOGY AND KINETICS OF METHYL- AND ETHYLMERCURY

Follow-up studies on children born to mothers exposed to methylmercury during the epidemic in Iraq suggested that the fetal brain is at particular risk (Marsh *et al.*, 1981, 1987). Consequently, intake limits set for methylmercury have to be low enough to prevent damage to the fetal brain. Daily intake limits range from 0.1 μg Hg $kg^{-1}$ $day^{-1}$ (Environment Protection Agency: EPA) to 0.47 μg $kg^{-1}$ $day^{-1}$ (WHO, 1990). The scarcity of data on ethylmercury explains the absence of similar assessment for ethylmercury. Reports from ethylmercury epidemics in China (Zhang, 1984) and Iraq (Damluji, 1962) listed only clinical manifestations without data on mercury in biological media. One way out of this impasse is to relate available data on the toxicity of ethylmercury to the toxicity of methylmercury and to give consideration to differences between both the kinetics of ethylmercury and methylmercury and the kinetics in adults and infants.

Mercury forms organometallic compounds in which one valence is occupied by an anion and the other by an organic radical. The choice of anion is dictated by physicochemical considerations concerning volatility or solubility, but the anion has no influence on the distribution or toxicity of the absorbed compound. Thus there is no difference between the distribution or toxicity of ethylmercury chloride or ethylmercury thiosalicylate (Suzuki *et al.*, 1973). Contrary to the anion, the size of the organic radical is the determinant of toxicity.

Table 1 summarizes the crucial differences between three organomercurials. With increasing size, the bond between mercury and the organic radical becomes less stable, the protective effect of the blood–brain barrier becomes more potent and the central nervous system (CNS) toxicity weakens. Contrary to the 19 days' clearance half-time of the methylmercury molecule in rats, phenylmercury decomposes with a half-time of 12 h and its access to the brain and toxicity to the CNS is in the category of inorganic mercury salts (Magos, 1981). Ethylmercury occupies a middle position between methyl- and phenylmercury. Compared with ethylmercury the passage of methylmercury across the blood–brain barrier is facilitated not only by its size but also by forming a complex

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Table 1—Relationship between the organic radicals of organomercurial compounds and toxicologically important characteristics

| | Methylmercury chloride | Ethylmercury chloride | Thimerosal | Phenylmercury acetate |
|---|---|---|---|---|
| Anion | Cl | Cl | COONa $C_6H_4S$ | $OCOCH_3$ |
| Metal | Hg | Hg | Hg | Hg |
| Organic radical | Methyl | Ethyl | Ethyl | Phenyl |
| Molecular weight | 251 | 265 | 405 | 337 |
| Weight of organ radical | 15 | 29 | 29 | 77 |
| Stability of Hg–C bond | Strong | Weaker | Weaker | Very weak |
| Potency of the blood–brain barrier | Weak | Moderate | Moderate | Strong |
| Neurotoxicity | Strong | Moderate | Moderate | None |

Table 2—Relationship between mercury in blood and brain and cerebellar granular layer damage in rats 10–12 days after five daily doses of methyl- or ethylmercury

| | Dose (mg Hg $kg^{-1}$) | | |
|---|---|---|---|
| Mercury conc. (μg $g^{-1}$) | Methylmercury 8 mg | Ethylmercury 8 mg | Ethylmercury 9.6 |
| *In blood* | | | |
| Total | 144 | 223 | 304 |
| Inorganic | 1.1 | 7.3 | 5.9 |
| *In brain* | | | |
| Total | 13.7 | 5.9 | 8.8 |
| Inorganic | 0.3 | 0.58 | 0.92 |
| Cerebellar granular layer damage | Sum of lobular scores (18 rats per group and 10 lobules per rat) | | |
| *In depth of lobules* | | | |
| Negative | 73 | 180 | 147 |
| 1+ | 57 | 0 | 33 |
| 2+ | 55 | 0 | 0 |
| 3+ | 5 | 0 | 0 |
| *In crest of lobules* | | | |
| Negative | 106 | 180 | 158 |
| 1+ | 54 | 0 | 22 |
| 2+ | 19 | 0 | 0 |
| 3+ | 1 | 0 | 0 |

with L-cysteine that is structurally similar to L-methionine. In this form, methylmercury is carried through the blood–brain barrier by an active transport system for neutral amino acids. Another transport mechanism, most likely diffusion, is about ten times slower than the active transport (Kerper *et al.*, 1992). Diffusion can operate for ethylmercury, although owing to its size at a slower rate.

Table 2 illustrates the influence of stability and the protective potency of the blood–brain barrier (Magos *et al.*, 1985). After identical doses, ethylmercury-treated rats had more mercury in the blood and less in the brain than their methylmercury counterparts, and the contribution of inorganic mercury was higher. The difference in blood and brain mercury concentrations means that the blood–brain concentration ratio established for methylmercury overestimates the brain mercury concentration for ethylmercury. The lower part of Table 2 demonstrates the meaning of these differences for CNS toxicity: that five daily doses of 8 mg Hg $kg^{-1}$ caused no damage in the cerebellar granular layer when it was given as ethylmercury, whereas methylmercury caused damage in 60% of the lobules. Even when the dose of ethylmercury was increased to 9.6 mg $kg^{-1}$, which is 86% of the lethal dose, <20% of the lobules were affected. Naturally the protective effect of the blood–brain barrier is restricted to the brain and does not cover other tissues such as dorsal root ganglia and kidneys.

There is some evidence that in humans also ethylmercury is less toxic than methylmercury. In the methylmercury epidemic in Iraq, death occurred in the group with a 4–5 μg $ml^{-1}$ blood mercury concentration (Bakir *et al.*, 1973), whereas in two deceased patients in the Iraq ethylmercury epidemic, the blood mercury concentration was 15 μg $ml^{-1}$ (Hilmy *et al.*, 1976). Thus these two victims went on with the consumption of ethylmercury-contaminated bread long after mercury in the blood passed the 5 μg $ml^{-1}$ mark.

## CLEARANCE HALF-TIME

Besides lower stability and inferior ability to cross the blood–brain barrier, faster clearance also moderates the toxicity of ethylmercury. For methylmercury the clearance

 

Table 3—Relationship between clearance half-time ($T_{1/2}$) and body burden ($b$) and blood mercury concentration at steady state

| | Formula | Methylmercury | Ethylmercury |
|---|---|---|---|
| Clearance half-time in days | $T_{1/2}$ | 50 | 18 |
| Daily loss in % of body burden | $b = \ln 2/T_{1/2}$ | 0.0139 | 0.039 |
| Body burden at steady state | Daily dose/$b$ | 72 7.2 | 26 2.6 |

Body burden and blood mercury concentrations at steady state for daily permissible intake limits

| Permissible daily intakes (μg Hg kg$^{-1}$ day$^{-1}$) | Body burden (μg Hg kg$^{-1}$) $T_{1/2}$ 50 days | Body burden (μg Hg kg$^{-1}$) $T_{1/2}$ 18 days | Blood Hg conc. (μg Hg l$^{-1}$ blood) $T_{1/2}$ 50 days | Blood Hg conc. (μg Hg l$^{-1}$ blood) $T_{1/2}$ 18 days |
|---|---|---|---|---|
| 0.1 (EPA) | 7.1 | 2.6 | 5.0 | 1.8 |
| 0.3 (ATSDR) | 21.5 | 7.7 | 15.0 | 5.4 |
| 0.4 (FDA) | 28.7 | 10.3 | 20.0 | 7.2 |
| 0.47 (WHO) | 33.5 | 12.1 | 23.5 | 8.4 |

half-time is given as 50 days in a person of 60 kg body weight (ATSDR, 1999). A report indicated that in four patients who were given plasma containing thimerosal, mercury cleared from red blood cells with 1 week half-time and at a slower rate from plasma (Suzuki *et al.*, 1973). Assuming a 40% haematocrit, these data indicate that mercury cleared from whole blood (in three patient with at least five estimations) with ca. 18 days' half time.

Table 3 illustrates the meaning of the difference between 50 and 18 days' half-times in relation to body burden at steady state, i.e. when, after prolonged exposure, daily excretion equals daily intake. It can be seen that at 50 days' half-time the daily loss of mercury in per cent of body burden is 2.8 times lower and the body burden is 2.8 times higher than at 18 days' half-time. The lower part of the table shows the effect of clearance on body burden and the blood concentration of mercury at steady state for different oral intake limits set for methylmercury by four agencies. The aim is to protect the fetal brain against the adverse effect of long-term (365 days or longer) meternal exposure. Differences between the limits of maternal exposure reflect differences in safety margins. The starting line for setting these limits was measured hair mercury concentrations, which were extrapolated to blood mercury and daily intake (ATSDR, 1999). Because hair mercury does not show short temporary fluctuations in input, it is implicit in the use of hair mercury for calculating daily intake or blood mercury that short-term fluctuations, e.g. rise in blood mercury after a meal, are of secondary importance.

## EXTRAPOLATION OF ADULT CLEARANCE HALF-TIME TO INFANTS

If infants handle ethylmercury less efficiently than adults do, they must have longer clearance half-time and higher blood mercury concentrations at the same level of intake. The argument against this possibility is that, unlike organ weights and volume of blood, which tend to increase in

Table 4—Comparison of observed (Ciba-Geigy, 1982) basal metabolic rates with metabolic rates allometrically extrapolated from a 60 kg body weight adult to infant body weights

| | Body weight (kg) | Basal metabolic expenditure (kcal/kg$^{-1}$ min$^{-1}$) Observed | Basal metabolic expenditure (kcal/kg$^{-1}$ min$^{-1}$) Extrapolated (deviation from observed) |
|---|---|---|---|
| Adult | 60 | 0.0187 | - |
| Infant | 10 | 0.0375 | 0.0320 (−15%) |
| | 9 | 0.0378 | 0.0330 (−13%) |
| | 8 | 0.0379 | 0.0342 (−10%) |
| | 7 | 0.0381 | 0.0356 (− 7%) |
| | 6 | 0.0383 | 0.0373 (− 3%) |
| | 5 | 0.0386 | 0.0394 (+ 2%) |
| | 4.5 | 0.0389 | 0.0407 (+ 5%) |
| | 4.0 | 0.0395 | 0.0421 (+ 7%) |
| | 3.5 | 0.0400 | 0.0438 (+10%) |

proportion to body weight, functions such as blood flow, ventilation and metabolic rates are related to the fractional power of body weight. This relationship is described by allometry (meaning 'of another measure') and the basic equation is

$$Y = aM^e \qquad (1)$$

where $Y$ is the variable and $M$ is the body mass in kg; scaling factor $a$ and exponent $e$ are empirically derived constants (National Academy of Sciences, 1987). When the rate of the function (basic metabolism, daily loss from body burden) is known in group one, the variable in another weight group can be extrapolated using

$$Y_2 = Y_1(M_1/M_2)^{0.3} \quad 0.0187 \qquad (2)$$

Table 4 shows that by inserting the 0.187 kcal kg$^{-1}$ min$^{-1}$ basic metabolic rate of a 60 kg man into Eqn. (2) the basic metabolic rate of infants ($Y$) can be extrapolated to be $Y = 0.187$ (60 kg/infant kg)$^{0.3}$. Differences between
0.0187

Table 5—Comparison of observed (Ciba-Geigy, 1984) clearance half-times ($T_{1/2}$) with allometrically extrapolated clearance half-times in different species (the daily loss of mercury in per cent of body burden is in parentheses)

| Species | Body weight (kg) | $T_{1/2}$ | | |
|---|---|---|---|---|
| | | Observed | Extrapolated | |
| | | | Eqn. (1) | Eqn. (2) |
| Human | 60 | 50 (0.0139) | 55 0.0126 (0.0124) | – |
| Macaque monkey | 4–6 | 22–26 (0.027–0.031) | 25–28 (0.025–0.028) | 22–25 (0.029–0.032) |
| Rat | 0.35 | 11–14 (0.050–0.063) | 12 (0.058) | 11 (0.063) |
| Mouse | 0.03 | 5–8 (0.087–0.138) | 5.6 (0.123) | 6 (0.110) |

observed (Ciba-Geigy, 1982) and extrapolated values are within 15%.

Table 5 compares observed and allometrically extrapolated clearance half-times ($T_{1/2}$) and the daily loss of mercury in per cent of body burden ($b$) in four species given methylmercury by using Eqn. (1): $b = 0.043(M)^{-0.3}$ and $T_{1/2} = 16.3(M)^{0.3}$. These equations give $b = 0.013$ and $T_{1/2} =$ 55 days for an adult of 60 kg body weight, which differ only slightly from $b = 0.014$ and $T_{1/2} = 50$ days (ATSDR, 1999) used in the following interspecies and adult to infant extrapolations (Eqn. (2)): $b = 0.014\ (60\ \text{kg}/M)^{0.3}$ and $T_{1/2} = 50(60\ \text{kg}/M)^{-0.3}$. Note that owing to the inverse relationship between $b$ and $T_{1/2}$ ($T_{1/2} = \ln 2/b$) in Eqn. (1) for $b$ and in Eqn. (2) for $T_{1/2}$, the exponent is negative.

Table 5 demonstrates that differences between observed half-times (FAO/WHO, 2000) and values given by the two allometric calculations for four species are within the acceptable range. Even a weight difference larger than the differences between the body weights of adults and infants does not invalidate the predictive use of allometry. The same equations were used for the extrapolation of $b$ and $T_{1/2}$ in infants.

Tables 6–8 justify the usefulness of allometric extrapolation for thimerosal. In the conversion of body burden to blood mercury concentration it was assumed that 5% of the body burden is in the blood compartment and that the blood volume is 7% of the body weight (ATSDR, 1999). This means that after the initial distribution phase the blood concentration is ca. 30% lower than the concentration in the whole body. Although during the initial phase of distribution the blood mercury concentration is higher, it moves towards this relationship (Kershaw *et al.*, 1980). The initial distribution phase is not significantly higher even for methylmercury, which has an active transport mechanism into brain. In Table 8 body weights for each infant age group were taken from published tabulations (Ciba-Geigy, 1984). The same source was used to calculate the time-dependent change in body weight from a measured value.

Table 6 gives the measured blood mercury concentrations in three groups of infants and compares these with extrapolated blood concentrations. In extrapolation, 50 and 18 days' half-times were used with and without allometry. Term neonates were on average 4.8-fold heavier than preterm neonates and therefore preterm neonates received a 4.8-fold higher dose than term neonates. Two and a half days after vaccination, their mercury concentration in blood was only 3.3 times higher than in term infants. Thus, contrary to the statement made by the authors of this report (Stajich *et al.*, 2000), preterm infants handled the ethylmercury load more efficiently than term infants did. In term neonates, owing to the combination of a low dose and the shortness of time between vaccination and blood sampling, both the half-times and their allometric extrapolation gave values near to the measured concentration. In the two other groups — the groups of preterm (Stajich *et al.*, 2000) and 2-month-old infants (Pichichero *et al.*, 2002) — the allometrically adjusted 18 days' clearance half-time gave the best prediction. This finding is confirmed in Table 7, which shows observed mercury concentration in 6-month-old infants 16 days after the last vaccination

Table 6—Measured and predicted blood mercury concentrations after vaccination in infants

| | Preterm neonates[a] | Term neonates[a] | 2-Month-old infants[b] |
|---|---|---|---|
| No. of infants | 15 | 5 | 20 |
| Body wt (kg) | 0.748 | 3.588 | 5.312 |
| Dose (μg Hg) | 12.5 | 12.5 | 50.0 |
| Dose (μg Hg kg$^{-1}$) | 16.7 | 3.48 | 9.41 |
| Blood (μg l$^{-1}$ Hg) at zero time | 11.7 | 2.44 | 6.59 |
| Measured blood Hg concentration (μg l$^{-1}$) | | | |
| 2.5 days after vaccination | 7.36 | 2.24 | – |
| 11 days after vaccination | – | – | 1.5 |
| Extrapolated blood Hg concentration (extrapolated/measured conc. ratio in parentheses) | | | |
| *Without allometry* | | | |
| $T_{1/2} = 50$ | 11.3 (1.54) | 2.35 (1.05) | 5.65 (3.77) |
| $T_{1/2} = 18$ | 10.61 (1.44) | 2.21 (0.99) | 4.31 (2.87) |
| *With allometry* | | | |
| $T_{1/2} = 50$ | 10.90 (1.48) | 2.25 (1.00) | 4.81 (3.21) |
| $T_{1/2} = 18$ | 8.17 (1.11) | 1.96 (0.88) | 2.74 (1.82) |

[a] Measured concentration from Stajich *et al.* (2000).
[b] Measured concentration from Pichichero *et al.* (2002).

Table 7—Calculated body burden of mercury in infants given 112.5 μg of Hg as thimerosal in the first 6 months of life and comparison of measured (Pichichero *et al.*, 2002) with calculated blood mercury concentration 16 days after the last vaccination (half-times were allometrically extrapolated to the weight of infants)

| Age (months) | Body wt (kg) | Hg dose (μg) | Body burden (μg) | | | |
|---|---|---|---|---|---|---|
| | | | Before dosing | | After dosing | |
| | | | $T_{1/2}$ = 50 | $T_{1/2}$ = 18 | $T_{1/2}$ = 50 | $T_{1/2}$ = 18 |
| 2 | 5.31 | 37.5 | | | 37.5 | 37.5 |
| 3 | 6.10 | – | 16.2 | 3.5 | – | – |
| 4 | 7.00 | 37.5 | 7.0 | 0.36 | 44.5 | 37.9 |
| 5 | 7.68 | – | 20.4 | 4.24 | – | – |
| 6 | 8.14 | 37.5 | 9.4 | 0.40 | 46.8 | 37.9 |
| *16 Days after last vaccination* | | | | | | |
| Measured blood Hg (μg $l^{-1}$) | | | | | 0.98 | |
| *Extrapolated* | | | | | | |
| Body burden (μg) | | | | | 31.1 | 12.2 |
| Body burden (μg Hg $kg^{-1}$) | | | | | 3.81 | 1.50 |
| Blood Hg (μg $l^{-1}$) | | | | | 2.70 | 1.07 |
| Calculated/measured blood Hg ratio | | | | | 2.8 | 1.09 |

Table 8—Predicted body burden and blood concentration of mercury in girls given 187.5 μg Hg as thimerosal in the first 6 months of life; the schedule of vaccination followed the pattern given by an Institute of Medicine document (2001)

| Age (months) | Body wt (kg) | Dose (μg) | Before dosing | | After dosing | |
|---|---|---|---|---|---|---|
| | | | μg Hg in body | μg Hg $l^{-1}$ blood | μg Hg in body | μg Hg $l^{-1}$ blood |
| 0 | 3.2 | 12.5 | | | 12.5 | 2.73 |
| 0.5 | 3.6 | | 3.2 | 0.62 | | – |
| 1 | 4. | – | 0.85 | 0.15 | | – |
| 1.5 | 4.35 | | 0.24 | 0.04 | | – |
| 2 | 4.7 | 62.5 | 0.07 | 0.01 | 63.2 | 9.41 |
| 2.5 | 5.0 | | 18.5 | 2.80 | | |
| 3 | 5.4 | – | 5.56 | 0.72 | | |
| 3.5 | 5.8 | | 1.72 | 0.29 | | |
| 4 | 6.2 | 50.0 | 0.54 | 0.06 | 50.5 | 5.70 |
| 4.5 | 6.5 | | 16.3 | 1.76 | | |
| 5 | 6.8 | – | 5.3 | 0.54 | | |
| 5.5 | 7.0 | | 1.8 | 0.18 | | |
| 6 | 7.2 | 62.5 | 0.60 | 0.06 | 63.1 | 6.13 |
| 6.5 | 7.4 | | 21.3 | 2.01 | | |
| 7 | 7.7 | – | 7.3 | 0.66 | | |
| 7.5 | 7.9 | | 2.5 | 0.22 | | |
| 8 | 8.2 | – | 0.87 | 0.07 | | |

(Pichichero *et al.*, 2002) and allometrically extrapolated concentrations after vaccination at 2, 4 and 6 months of age.

Table 8 follows the vaccination schedule given in an Institute of Medicine document (2001). This table shows that mercury concentration in blood never exceeds the ATSDR, FDA or WHO limits but it exceeds the 5.0 μg $l^{-1}$ EPA limit in decreasing order after the second, third and fourth vaccinations. Even after the highest blood mercury concentration of 9.4 μg $l^{-1}$, the level decreased below 5 μg $l^{-1}$ within 7 days. Apart from clearance, an increase in body weight influenced the concentration by there being dilution in a larger volume.

Peak concentrations, even from highly toxic doses, are not an absolute measure of CNS toxicity. Table 9 shows that when rats were given 8 mg Hg $kg^{-1}$ as methylmercury for 6 days, all of them developed cerebellar damage. However, when DMSA (dimercaptosuccinic acid, a potent depletor of mercury stores) was given 3–5 days after the last exposure day, the DMSA-treated rats had less mercury in the brain and developed significantly less damage (Magos *et al.*, 1978). A similar observation was made on a patient who had 14 μg $ml^{-1}$ blood mercury concentration after the ingestion of 41 mg $kg^{-1}$ mercury as thimerosal. (Pfab *et al.*, 1996).

Table 9—Effect of dimercaptosuccinic acid (DMSA, which depletes mercury stores) on the brain concentration of mercury and cerebellar damage in rats given 8 mg $kg^{-1}$ mercury as methylmercury for 6 days

| Days after last dose | Brain mercury concentration ($\mu g\ g^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | No DMSA | DMSA given on days in parentheses | | | | |
| 1 | 18.7 | | | | | |
| 3–4 | 21.2 | 15.5(1,2), 17.3(2) | | | | |
| 5–7 | 21.0 | 9.6(1,2,3), 8.9(2,3,4), 12.6(3,4,5) | | | | |
| 8–10 | 23.6 | 8.1(1,2,3), 7.3(2,3,4), 8.9(3,4,5), 8.1(4,5,6), 8.5(5,6,7) | | | | |
| | DMSA | No. of rats | Cerebellar granular layer damage (% of rats) | | | |
| | | | 1+ | 2+ | 3+ | 4+ |
| 9–20* | No | 12 | 0 | 37 | 26 | 37 |
| | yes | 19 | 37 | 26 | 37 | 0 |

* Three days of treatment with DMSA started 3–5 days after the last methylmercury dose.

## CONCLUSIONS

(i) Decomposition (splitting of the mercury–carbon bond) is a detoxification process for CNS toxicity. Because ethylmercury is decomposed faster than methylmercury, the risk of brain damage is less for ethyl- than for methylmercury, even if access of the intact molecules to the brain were the same.

(ii) Access of ethylmercury to the brain is below the access of methylmercury, partly because passage through barriers favours the smaller molecule but mostly because methylmercury is actively transported through the blood–brain barrier. Thus the blood–brain mercury concentration ratio established for methylmercury will overestimate mercury in the brain after exposure to ethylmercury.

(iii) Mercury clears from the body faster after the administration of ethylmercury than after the administration of methylmercury.

(iv) Because metabolic rates (basic metabolism, rate of loss from the body burden) are related to the fractional power (<1 and mostly 0.3) of body weight (allometric relationship), mercury clears from the infant body faster than from the adult body.

(v) Based on the above points, the input limits and corresponding blood mercury concentrations suggested for methylmercury underestimate the safe exposure range for ethylmercury.

## REFERENCES


American Academy of Pediatrics and Public Health Service. 1999. Thimerosal in vaccines: a joint statement. *Morb. Mortal. Weak. Rep.* **48**: 563–565.

ATSDR (Agency for Toxic Substances and Disease Registry). 1999. Toxicological profile for mercury (update). US Department of Health and Human Services: Atalanta, GA.

Bakir F, Damluji SF, Amin-Zaki L, Murthada M, Khalidi A, Al-Rawi NY, Tikriti S, Dhahir HI, Clarkson TW, Smith JC, Doherty RA. 1973. Methylmercury poisoning in Iraq. *Science* **181**: 230–241.

Ball LK, Ball R, Pratt KD. 2001. An assessment of thiomersal use in childhood vaccines. *Pediatrics* **107**: 1147–1153.

Ciba-Geigy. 1982. *Geigy Scientific Tables 1982*, vol. 1. Ciba-Geigy: Basle; 228–230.

Ciba-Geigy. 1984. *Geigy Scientific Tables 1984*, vol. 3. Ciba-Geigy: Basle; 298–300.

Damluji S. 1962. Mercurial poisoning with the fungicide Granosan M. *J. Fac. Med. (Baghdad)* **4**: 83–103.

FAO/WHO. 2000. Methylmercury. In *Safety Evaluation of Certain Food Additives and Contaminants*. WHO Food Additives Series 44. World Health Organization: Geneva; 326–337.

Freed G, Andreae MC, Cowan AE, Katz SL. 2002. The process of public policy formulation: the case of thimerosal in vaccines. *Pediatrics* **109**: 1153–1159.

Hilmy MI, Rahim SA, Abbas AH. 1976. Normal and lethal mercury levels in human beings. *Toxicology* **6**: 155–159.

Institute of Medicine. 2001. *Immunization Safety Review. Thimerosal-containing Vaccines and Neurodevelopmental Disorders*, Stratton K, Gable A, McCormick MC (eds). National Academy Press: Washington, DC.

Kerper LA, Ballatori N, Clarkson TW. 1992. Methylmercury transport across blood–brain barrier by an amino acid carrier. *Am. J. Physiol.* **262**: R761–R765.

Kershaw TG, Clarkson TW, Dhahir PH. 1980. The relationship between blood levels and dose of methylmercury in man. *Arch. Environ. Health* **35**: 28–36.

Magos L. 1981. Metabolic factors in the distribution and half time of mercury after exposure to different mercurials. In *Industrial and Environmental Xenobiotics*, Gut I, Cikrt M, Plaa G (eds). Springer Verlag: Berlin; 1–15.

Magos L, Peristianis GC, Snowden RT. 1978. Postexposure preventive treatment of methylmercury intoxication in rats with dimercaptosuccinic acid. *Toxicol. Appl. Pharmacol.* **45**: 463–475.

Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. 1985. The comparative toxicology of ethyl- and methylmercury. *Arch. Toxicol.* **57**: 260–267.

Marsh DO, Myers GJ, Clarkson TW, Cox C, Amin-Zaki L, Tikriti S, Majeed MA, Dabbagh AR. 1981. Dose–response relationship for human fetal exposure to methylmercury. *Clin. Toxicol.* **18**: 1311–1318.

Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Tikriti S. 1987. Fetal methylmercury poisoning. *Arch. Neurol.* **44**: 1017–1022.


 

National Academy of Sciences. 1987. *Pharmacokinetics in Risk Assessment*. National Academy Press: Washington, DC; 65–79.

Pfab R, Muckter H, Roider G, Zilker T. 1996. Clinical course of severe poisoning with thiomersal. *J. Toxicol. Clinical Toxicol.* 34: 453–460.

Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. 2002. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. *Lancet* 360: 1737–1741.

Pless R, Risber JF. 2000. Mercury, infant neurodevelopment, and vaccination [Editorial]. *J. Pediatr.* 136: 571–573.

Stajich GV, Lopez GP, Harry SW, Sexson WR. 2000. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. *J. Pediatr.* 136: 679–681.

Suzuki T, Takemoto, T-I, Kashiwazaki H, Miyama T. 1973. Metabolic fate of ethylmercury salts in man and animals. In *Mercury, Mercurials and Mercaptans*, Miller MW, Clarkson TW (eds). Charles C. Thomas: Springfield, IL; 209–240.

WHO. 1990. *Environmental Health Criteria 101. Methylmercury.* World Health Organization: Geneva.

Zhang J. 1984. Clinical observations in ethyl mercury chloride poisoning. *Am. J. Indust. Med.* 5: 251–258.

because it could help displace the goal-less, progress driven, never-happy medicine that grew out of [its] embrace of modernism." We need to understand "the social meaning of medicine and health care," he declared, "and the relationship of medicine to the cultures of which it is a part." In this regard, J. Kirby[10] of Australia also made some relevant comments (concerning the need to slow the headlong rush of modern medicine),

"My hope is that it won't be the epitaph of our generation that people will say: 'Here was a community which developed the most amazing, dazzling fields of science and yet proved themselves so indifferent or incompetent, that they didn't address the serious social and ethical consequences of what they were up to.'"

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REFERENCES

1. Hart JT. Two paths for medical practice. *Lancet*. 1992;340:772–775
2. Callahan D. *False Hopes*. New Brunswick, NJ: Rutgers University Press; 1999
3. Kleinke JD. *Oxymorons: The Myth of a U. S. Health Care System*. New York, NY: Jeffrey-Bass; 2001
4. Winslow R. Infant health problems cost business billions. *Wall Street Journal*. May 5, 1992
5. Jobe AH. Predictors of outcomes in preterm infants: which ones and when? *J Pediatr*. 2001;138:153–156
6. Culver G, Fallon K, Londner R, et al. Informed decisions for extremely low-birth-weight infants [letter]. *JAMA*. 2000;283:3201
7. Lorenz JM, Paneth N, Jetton JR, den Ouden L, Tyson JE. Comparison of management strategies for extreme prematurity in New Jersey and The Netherlands: outcomes and resource expenditure. *Pediatrics*. 2001;108:1269–1274
8. Thompson LA, Goodman DC, Little GA. Is more neonatal intensive care always better? Insights from cross-national comparison of reproductive care. *Pediatrics*. 2002;109:1036–1043
9. Sheldon T. Dutch doctors change policy on treating preterm babies. *BMJ*. 2001;322:1383
10. What rules for embryology? [editorial]. *Manchester Guardian Weekly*. February 7, 1981

## Thimerosal and Autism?

Concern has been expressed over the possibility that the mercury-containing compound thimerosal in vaccines may cause autism.[1–4] Thimerosal is sodium ethylmercury thiosalicylate, an organic compound of ethyl mercury, included in certain vaccines to protect multiple dose ampules from bacterial and fungal contamination. Mercury in sufficient dose is neurotoxic, and probably more toxic in the immature brain. It is reasonable to ask whether thimerosal in childhood vaccine increases risk of chronic childhood neurologic disability and specifically of autism. The available data with which to address the question are very limited and largely inferential. Most of the information we have about mercury toxicity is related to exposure to methyl rather than ethyl mercury.

Bernard et al[1] offered an hypothesis that autism is an expression of mercury toxicity resulting from thimerosal in vaccines. They base this hypothesis on their views[2] that the clinical signs of mercury toxicity are similar to the manifestations of autism, that the onset of autism is temporally associated with immunization in some children, that the recent increase in diagnosis of autism parallels exposure to thimerosal, and that there are higher levels of mercury in persons with than without autism.

This review will examine these issues and others to ask whether, according to evidence now available, thimerosal is a probable cause of autism. We will not discuss which, if any, of the differing guidelines designed to limit exposure to mercurials is appropriate for deciding whether thimerosal in vaccines is in all regards safe for children. Our focus is on a narrower but important question: whether current evidence indicates that mercury at any known dose, form, duration, age, or route of exposure leads to autism.

### ARE THE CLINICAL MANIFESTATIONS OF AUTISM SIMILAR TO THOSE OF RECOGNIZED MERCURY TOXICITY?

Bernard et al[1] present a table listing ~95 clinical findings they consider to be shared by autism and mercury poisoning. Their table does not distinguish typical and characteristic manifestations of either disorder from the rare, unusual, and highly atypical.

In mercury poisoning, the characteristic motor findings are ataxia and dysarthria (Table 1).[5,6] These signs, along with tremor, muscle pains, and weakness, are noted on relatively high-dose exposure, acute or chronic. In 3 Romanian children accidentally exposed to ethyl mercury in a fungicide, these same symptoms were prominent.[7] The outcome of fetal methyl mercury poisoning in severe form also included spasticity.[8] In contrast, in autism, the only common motor manifestations are repetitive behaviors (stereotypies) such as flapping, circling, or rocking. Persons with Asperger syndrome may be clumsy, and hypotonia has been noted in some infants with autism; the frequency of clumsiness and hypotonia in autism spectrum disorders is not established. No other motor findings are common in autism, and indeed the presence of ataxia or dysarthria in a child whose behavior has autistic features should lead to careful medical evaluation for an alternative or additional diagnosis.

The most characteristic sensory finding of mercury poisoning is a highly specific bilateral constriction of visual fields.[5,6,9] With lesser exposure there may be compromise of contrast sensitivity.[10,11] In addition, there may be paresthesias or, in infants, erythema and pain in hands and feet because of peripheral neuropathy. In autism, decreased responsiveness to pain is sometimes observed along with hypersensitivity to other sensory stimuli, including hyperacusis. The "sensory defensiveness" of autism seems to reflect altered sensory processing within the brain rather than peripheral nerve involvement.[12–14]

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TABLE 1. Characteristic Findings in Autism and in Mercury Poisoning

| | Autism | Mercurism |
|---|---|---|
| Motor | Stereotypies | Ataxia, dysarthria |
| Vision | No abnormality | Constricted visual fields |
| Speech | Delay, echolalia | Dysarthria |
| Sensory | Hyper-responsiveness | Peripheral neuropathy |
| Psychiatric | Socially aloof, insistence on sameness | Toxic psychosis; in mild cases, nonspecific depression, anxiety |
| Head size | Large | Small |

Other signs that may appear in children with chronic mercury toxicity, such as hypertension,[15] skin eruption,[16] and thrombocytopenia,[17] are seldom seen in autism.

In relatively mild mercurism in persons without characteristic motor or sensory changes, psychiatric symptomatology may be absent, and if present is nonspecific, with findings such as depression, anxiety, and irritability.[18–20] There may be impairment of recent memory. Even for individuals with known elevated postmortem levels of mercury in brain, it may be impossible to conclude whether the nonspecific psychiatric findings they demonstrated in life were the result of mercury toxicity.[21]

When severe mercury poisoning occurs in prenatal life or early infancy, head size tends to be small and microcephaly is common.[22] Prenatal exposure to other neurotoxins—lead, alcohol, and polychlorinated biphenyls, for example—also predispose to decreased head size. In contrast, in autism increasing evidence indicates that head size[23–25] and, as measured by volumetric magnetic resonance imaging, brain size[26,27] tends to be larger than population norms.

At sufficient dose mercury is indeed a neurotoxin, but the typical clinical signs of mercurism are not similar to the typical clinical signs of autism.

## ONSET OF AUTISM SYMPTOMS AFTER IMMUNIZATIONS

Evaluation of causation cannot depend on temporal association as reflected by anecdotal observations of selected instances in which a relatively uncommon outcome such as autism is noted after a common childhood exposure such as immunization. Only rigorous methods that attempt to include all instances of both exposure and outcome can provide evidence of association, and association is necessary but not sufficient to establish causation.

Age of onset of symptoms can be highly misleading as an indicator that some environmental event has caused or precipitated a disorder. Even single gene disorders may have a period of apparently normal development (~1.5 years in Rett syndrome, 45 years in Huntington's chorea) before symptoms begin. The onset of clinically recognizable signs and symptoms in Rett and Huntington syndromes does not require an environmental "second hit." In Rett syndrome, the mutation causes previously apparently normal children to lose acquired developmental milestones after 1 years old to 2 years old, with a phase during which they may present behaviors consistent with autism. This disorder can also have its clinically apparent onset soon after the completion of immunizations, but Rett syndrome is known to be determined by a single genetic mutation that produces failure in the normal program of brain development. If we did not understand its genetic basis, we might suspect that Rett syndrome was attributable to environmental factors including immunization. The situation for autism is still unknown, but the onset of signs in the second year of life does not prove (nor disprove) a role for environmental factors in etiology.

## INCREASE IN DIAGNOSIS OF AUTISM IN PARALLEL WITH INTRODUCTION OF MERCURY-CONTAINING VACCINES

There has clearly been a broadening of the criteria for autism, better case-finding, increased awareness by clinicians and by families, and an increase in referrals of children for services as it has become recognized that early treatment improves life for the child and family.[28,29] Whether the sum of these is sufficient to account for the more frequent diagnosis of autism is a matter of contention and is properly settled by careful research.

If, for the sake of discussion, we assume there was a true increase in the occurrence of autism in the 1990s, is exposure to thimerosal the only or the best hypothesis to explain the increase? There have been many changes in life in industrialized countries during the last decades, including changes in many environmental exposures and aspects of medical care that could be considered for their biological plausibility as contributors to autism occurrence or severity.

## MERCURY LEVELS IN AUTISTIC PERSONS

Bernard et al[2] state that "elevated mercury has been detected in biological samples of autistic patients," but unfortunately do not provide references. Aschner and Walker[30] found no paper published in the peer-reviewed literature that reported an abnormal body burden of mercury, or an excess of mercury in hair, urine, or blood. The one paper that sought a relationship between autism and mercury levels in hair did not observe such an association.[31] We did not find evidence that chelation therapy has led to improvement in children with autism.

## NEUROPATHOLOGY

A substantial literature describes the neurotoxicity of methyl mercury but relatively little is known about the impact of ethyl mercury on the nervous system, especially with repeated low-dose exposure.

The passage of methyl mercury across the blood-brain barrier is facilitated by an active transport mechanism, whereas the passage of ethyl mercury into the brain does not have such a transport system and is further hindered by its larger molecular size and faster decomposition.[32] At equivalent doses, higher levels of mercury have been found in the blood and less in brain following administration of ethyl mercury than methyl mercury.[33] These findings support the observation that the risk of toxicity from ethyl mercury is overestimated by comparison with the risk of intoxication from methyl mercury.[34] Ethyl mercury exposure has been reported to be more likely than methyl mercury to produce lesions of the spinal cord, skeletal muscle, and myocardium.[8]

The effects of mercurial compounds are influenced by dose and duration of exposure and by maturational stage.

Studies in experimental animals exposed postnatally to ethyl mercury indicate patchy damage in the cerebellar granule cell layer, while methyl mercury produced a diffuse abnormality.[35] Methyl mercury exposure has been reported to disrupt neuronal migration primarily in the motor cortex[36] and in the cerebellar granule cell layer.[37] In humans with massive exposure to mercurials resulting in death, brains showed severe atrophy and gliosis of calcarine cortex, as well as diffuse neuronal loss and gliosis of the auditory, motor and sensory cortices, and extensive cerebellar atrophy.[38]

The most extensive pathologic studies of the brain in mercury poisoning followed methyl mercury exposure resulting from contaminated seafood in Japan and from contaminated bread in Iraq. Microscopic findings in these brains included decreased numbers of neurons and increased numbers of glial cells and macrophages throughout the cortex, as well as loss of granule cells and irregularity of the Purkinje cell layer in the cerebellum. In 2 Iraqi infants exposed prenatally to methyl mercury there was a simplified gyral pattern, short frontal lobe, and reduction in white matter volume, along with derangement and lack of definition of the cortical layers and heterotopic neurons in cerebrum and cerebellum.[39]

Thus, in both prenatally and postnatally exposed brain, methyl mercury resulted in neuronal cell loss and increased gliosis in the cerebral cortex, in some adults marked atrophy of the calcarine cortex, and atrophy of the cerebellum with consistent loss of granule cells and relative sparing of Purkinje cells. The weight or volume of the mercury-exposed brains has not been presented, but the atrophy associated with neuronal loss and in the infant cases the reduced white matter volume suggest that these brains were likely to be reduced in size.

In ethyl mercury toxicity in children, nerve cell loss was widely present but most marked in calcarine cortex, and there was diffuse proliferation of glia, demyelination of ninth and tenth cranial nerve roots, and atrophy of the cerebellar granule cell layer with relative sparing of Purkinje cells.[8]

In contrast, examined at autopsy, brains of autistic persons are commonly enlarged both by weight[40] and volume.[26] Larger head circumference and enlargement seen on volumetric magnetic resonance imaging studies in autism have been noted above. There have been no reports of significant cerebral cortical neuronal loss or calcarine atrophy in autism. The most frequently reported findings in the autistic forebrain have been unusually small, closely packed neurons and increased cell packing density in portions of the limbic system, consistent with curtailment of development of this circuitry.[40]

Age-related abnormalities have been observed in the deep cerebellar nuclei and inferior olivary nucleus of the brainstem in autism. The most consistent finding in the neuropathology of autism is reduction in Purkinje cells in the cerebellum, primarily in the posterior inferior hemispheres.[41–43] Involvement of granule cells has rarely been reported. In contrast, mercury-exposed brains have shown significant and consistent damage to the cerebellar granule cell layer with relative preservation of Purkinje cells.

Thus, there seem to be major differences in the neuroanatomic findings in autism as compared with those in mercury toxicity.

## IN HUMAN POPULATIONS EXPOSED TO MERCURY, DID AUTISM INCREASE?

In the first half of the 20th century, mercury was a constituent of medications administered to treat worm infestations and teething pain. Use of these compounds was associated with illness in young children, affecting chiefly those 8 months old to 2 years old. These infants showed photophobia, anorexia, skin eruption, and bright pink color of hands and feet, which peeled and were painful.[44] This condition, called "pink disease" or acrodynia, was relatively common, and the cause of 103 deaths in England and Wales in 1947.[45] Survivors were not described to have behavioral disorders suggestive of autism.

In the 1950s in Minamata and in the 1960s in Niigata, Japan, there were epidemics of methyl mercury poisoning resulting from discharge of industrial wastes into coastal waters, with consumption of contaminated fish by humans. Heavy prenatal exposure resulted in low birth weight, microcephaly, profound developmental delay, cerebral palsy, deafness, blindness, and seizures.[6,46] Affected adults experienced impairments of speech, constriction of visual fields, ataxia, sensory disturbance, and tremor.

Was autism recognized with higher frequency in Japanese children in the period of these toxic outbreaks or soon after it, especially in those born in the regions affected by the tragic poisonings? Japanese reports in the English language do not indicate that Japanese clinicians thought so. Comparable in earlier periods, the rates of autism were higher as reported in Japan in the 1980s than in studies from other countries.[47–49] This difference was attributed by Japanese authors to broader diagnostic criteria and excellent ascertainment.[50] Definitions and methods of ascertainment were widely different in different studies, so comparisons are difficult. A study in Fukushima-ken[51] is described here in some detail because it provides an example of the issues faced by studies of prevalence during this period and includes an analysis by year of birth in an area not far distant from Niigata. In this study, conducted in 1977, the authors attempted to evaluate all children with autism 18 years old or less who were born in the province in 1960 through 1977. They ascertained cases by sending a letter and questionnaire to 2233 institutions to find children with "autistic behavior," not further defined. Responses were received from 72.6% of the institutions, which covered 38% to 40% of children in the province. How responding institutions differed from those not responding is not stated. The autism prevalence estimates reported included children in the responding institutions in the numerator, and all children in the area in the denominator. If the nonresponding institutions had affected children in their care, and if there were changes over the period of the study that might influence recruitment of affected children at competing institutions, such changes could markedly influence the result. Based on their final diagnosis, there were more children with "autistic mental retardation" than with "early infantile autism," but no information is provided on the basis for this distinction, nor on birth year patterns for the former group.

The authors of the Fukushima-ken study[51] reported higher rates of autism in children born between 1966 and 1974 than in births 1960 through 1965 or after 1975. The authors considered that the reason for the lower rates of autism in children born before 1966 "was probably that autistic children had become older, lost the unique feature[s] of young autistic children and had been overlooked." This suggests that procedures for locating older subjects and criteria for diagnosis were not appropriate for all of the wide age span evaluated. For children born in the last years of the study, the low rates of autism surely entail severe undercounts as these children were 3 years old or less at the time of ascertainment. Although this study might have tested the question as to whether autism was more frequent near to outbreaks of mercury poisoning, methodologic problems potentially invalidate the time trend analysis, and the short follow-up for the most recent birth years means that no conclusions can be drawn regarding children born 1974 or later.

Studies that followed victims of high-dose acute or chronic mercury poisoning resulting from contaminated foods in Iraq, Pakistan, Guatemala, and Ghana have not reported manifestations suggestive of autism in survivors. In contrast, many of these survivors had clinical signs such as persisting ataxia and dysarthria that are seldom seen in autism.

An unpublished retrospective study was noted by the Institute of Medicine's Immunization Safety Review Committee.[52] As described in a Canadian Communicable Disease Report,[53] this study examined 10 years of data from a large database derived from 7 health maintenance organizations that covered ~2.5% of the United States population. A weak but statistically significant (relative risk ratio <2.0) association was found between measures of cumulative exposures to thimerosal and the presence of speech delay and attention-deficit/hyperactivity disorder, but not autism. There were many limitations of this analysis and its ability to identify bias and confounding. A second unpublished screening study did not confirm the findings of the first. Although far from definitive, these studies represent the only direct investigation to date of a possible association of thimerosal exposure with autism. Neither study observed such an association.

Two studies have examined neurologic and psychologic function in young children associated with lower dose but repeated dietary exposure to methyl mercury. In the Faeroe Islands, exposure was via consumption of marine fishes and mammals (whales). In the Faeroes, there may have been additional toxins including polychlorinated biphenyls and perhaps others.[54,55] The Faeroe study of 428 to 900 children at 7 years old observed an association of mercury levels in cord blood or maternal hair with impaired performance in tests of attention, memory for visuospatial information, the Boston Naming Test, fine motor function, and verbal learning.[56,57] In contrast, in the Sechylles study of >700 children, exposure was to marine fish only, and boys with higher levels of hair mercury performed better on some tests, including the Boston Naming Test and 2 tests of visual motor coordination.[59,60] The authors considered their enhanced performance might be related to beneficial effects of constituents other than mercury in fish. Myers et al[54] have discussed sources of difference in the results of these studies.

The Faeroe and Seychelles studies were probably large enough to detect a substantial but not a minor increase in autism, if it was present. Neither study was designed to investigate an association of mercury exposure with autism, but autism in all but its milder forms produces fairly striking behavioral aberration in young children. Were the endpoints examined appropriate for identification of children with autism? The Faeroe study included little behavioral assessment. Based on experience in lead toxicity studies, the Seychelles study used the Child Behavior Checklist overall rating at 66 months and 96 months. Testing at 66 months included Checklist subscales related to withdrawal, anxiety, and problems in social function, attention, and thought. The Child Behavior Checklist is not ideally sensitive for recognition of autism, but would probably identify the majority of affected children.[60] Myers et al,[54] reviewing nearly 50 years of research on mercury exposure and 27 years experience in human neurotoxicity of methyl mercury, concluded, "Our research has not identified any adverse associations between [methyl mercury] exposure from fish consumption and clinical symptoms or signs."

## CONCLUSION

Thimerosal is being eliminated from the vaccines used in routine infant immunization programs in the United States and Canada. If thimerosal was an important cause of autism, the incidence of autism might soon begin to decline. One can hope but not expect that that will happen; time will tell.

Mercury poisoning and autism both affect the central nervous system but the specific sites of involvement in brain and the brain cell types affected are different in the two disorders as evidenced clinically and by neuropathology. Mercury also injures the peripheral nervous system and other organs that are not affected in autism. Nonspecific symptoms such as anxiety, depression, and irrational fears may occur both in mercury poisoning and in children with autism, but overall the clinical picture of mercurism—from any known form, dose, duration, or age of exposure—does not mimic that of autism. No case history has been encountered in which the differential diagnosis of these 2 disorders was a problem. Most important, no evidence yet brought forward indicates that children exposed to vaccines containing mercurials, or mercurials via any other route of exposure, have more autism than children with less or no such exposure.

Continuing vigilance is necessary regarding the safety of vaccines, as is open-minded evaluation of new evidence. However, such evidence must be of sufficient scientific rigor to provide a responsible basis for decisions that influence the safety of children. When information is incomplete, as it is at present for thimerosal-autism questions, a balancing must be made of risks posed by vaccine constituents and the benefits of disease prevention achieved by keeping immunizations widely available. On the basis of current evidence, we consider it improbable that thimerosal and autism are linked.

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## REFERENCES

1. Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. *Med Hypotheses*. 2001;56:462–471
2. Bernard S, Enayati A, Roger H, Binstock T, Redwood L. The role of mercury in the pathogenesis of autism. *Mol Psychiatry*. 2001;7:S42–S43
3. Kidd PM. Autism, an extreme challenge to integrative medicine. Part 1: the knowledge base. *Altern Med Rev*. 2002;7:292–316
4. http://www.safeminds.org
5. Kark RAP, Poskanzer DC, Bullock JD, Boylen G. Mercury poisoning and its treatment with n-acetyl-D, L-penicillamine. *N Engl J Med*. 1971; 285:10–16
6. Eto K. Minamata disease. *Neuropathology*. 2000;20:S14–S19
7. Cinca I, Dumitrescu I, Onaca P, Serbanescu A, Nestorescu B. Accidental ethyl mercury poisoning with nervous system, skeletal muscle, and myocardium injury. *J Neurol Neurosurg Psychiatry*. 1979;43:143–149
8. Amin-Zaki L, Majeed MA, Greenwood MR, Elhassani SB, Clarkson TW, Doherty RA. Methylmercury poisoning in the Iraqi suckling infant: a longitudinal study over five years. *J Appl Toxicol*. 1981;1:210–214
9. Korogi Y, Takahashi M, Hirai T, et al. Representation of the visual field in the striate cortex: comparison of MR findings with visual field deficits in organic mercury poisoning (Minamata disease). *Am J Neuroradiol*. 1997;18:1127–1130
10. Altmann L, Sveinsson K, Kramer U, et al. Visual functions in 6-year-old children in relation to lead and mercury levels. *Neurotoxicol Teratol*. 1998;20:9–17
11. Lebel J, Mergler D, Lucotte M, et al. Evidence of early nervous system dysfunction in Amazonian populations exposed to low-levels of methylmercury. *Neurotoxicology*. 1996;17:157–167
12. Wong V, Wong SN. Brainstem auditory evoked potential study in children with autistic disorder. *J Autism Dev Disord*. 1991;21:329–340
13. Gomot M, Giard MH, Adrien JL, Barthelemy C, Bruneau N. Hypersensitivity to acoustic change in children with autism: electrophysiological evidence of left frontal cortex dysfunctioning. *Psychophysiology*. 2002;39: 577–584
14. O'Neill M, Jones RS. Sensory-perceptual abnormalities in autism: a case for more research? *J Autism Dev Disord*. 1997;27:283–293
15. Torres AD, Ashok AN, Hardiek ML. Mercury intoxication and arterial hypertension: report of two patients and review of the literature. *Pediatrics*. 2000;105(3):34. Available at www.pediatrics.org/cgi/content/full/105/3/e34
16. Boyd AS, Seger D, Vannucci S, Langley M, Abraham JL, King LE Jr. Mercury exposure and cutaneous disease. *J Am Acad Dermatol*. 2000;43: 81–90
17. Fuortes LJ, Weisman DN, Graeff ML, Bale JF Jr, Tannous R, Peters C. Immune thrombocytopenia and elemental mercury poisoning. *J Toxicol Clin Toxicol*. 1995;33:449–455
18. Maghazaji HI. Psychiatric aspects of methylmercury poisoning. *J Neurol Neurosurg Psychiatry*. 1974;37:954–958
19. Ross WD, Sholiton MC. Specificity of psychiatric manifestations in relation to neurotoxic chemicals. *Acta Psychiatr Scand*. 1983;67(suppl 303):100–104
20. Powell TJ. Chronic neurobehavioural effects of mercury poisoning on a group of Zulu chemical workers. *Brain Inj*. 2000;14:797–814
21. Wheatley B, Barbeau A, Clarkson TW, Lapham LW. Methylmercury poisoning in Canadian Indians—the elusive diagnosis. *Can J Neurol Sci*. 1979;6:417–422
22. Ramirez G, Cruz MC, Pagulayan O, Ostrea E, Dalisay C. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infants' hair. *Pediatrics*. 2000;106: 774–781
23. Davidovitch M, Patterson B, Gartside P. Head circumference measurements in children with autism. *J Child Neurol*. 1996;111:389–393
24. Lainhart JE, Piven J, Szorek M, et al. Macrocephaly in children and adults with autism. *J Am Acad Child Adolesc Psychiatry*. 1997;36:282–290
25. Stevenson RE, Schroer RJ, Skinner C, Fender D, Simensen RJ. Autism and macrocephaly. *Lancet*. 1997;349:1744–1745
26. Courchesne E, Karns BS, Davis HR, et al. Unusual brain growth patterns in early life in patients with autistic disorder, an MRI study. *Neurology*. 2001;57:245–254
27. Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N. Effects of age on brain volume and head circumference in autism. *Neurology*. 2002;59: 175–183
28. Wing L, Potter D. The epidemiology of autistic spectrum disorders: is the prevalence rising? *Ment Retard Dev Disabil Res Rev*. 2002;8:151–161
29. Croen LA, Grether JK, Hoogstrate J, Selvin S. The changing prevalence of autism in California. *J Autism Dev Disord*. 2002;32:207–215
30. Aschner M, Walker SJ. The neuropathogenesis of mercury toxicity. *Mol Psychiatry*. 2002;7:S40–S41
31. Wecker L, Miller SB, Cochran SR, Dugger DL, Johnson WD. Trace element concentrations in hair from autistic children. *J Ment Defic Res*. 1985;29:15–22
32. Kerper LE, Ballatori N, Clarkson TW. Methylmercury transport across the blood-brain barrier by an amino acid carrier. *Am J Physiol*. 1992;262: R761–R765
33. Suzuki T, Miyama T, Toyama C. The chemical form and bodily distribution of mercury in marine fish. *Bull Environ Contam Toxicol*. 1973;10: 347–355
34. Magos L. Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical products. *J Appl Toxicol*. 2001;21:1–55
35. Magos L. The comparative toxicology of ethyl- and methylmercury. *Arch Toxicol*. 1985;57:260–267
36. Kakita A, Inenaga C, Sakamoto M, Takahashi H. Neuronal migration disturbance and consequent cytoarchitecture in the cerebral cortex following transplacental administration of methylmercury. *Acta Neuropath*. 2002;104:409–417
37. Sass JB, Haselow DT, Silbergeld EI. Methylmercury-induced decrement in neuronal migration may involve cytokine-dependent mechanisms: a novel method to assess neuronal movement in vitro. *J Toxicol Sci*. 2001;63:74–81
38. Nierenberg DW, Nordgren RE, Chang MB, et al. Delayed cerebellar disease and death after accidental exposure to dimethylmercury. *N Engl J Med*. 1998;338:1672–1676
39. Choi BH, Lapham LW, Amin-Zaki L, Saleem T. Abnormal neuronal migration, deranged cerebral cortical organization, and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmer-

cury poisoning in utero. *J Neuropathol Exp Neurol.* 1978;37:719–733
40. Kemper TL, Bauman ML. Neuropathology of infantile autism. *Mol Psychiatry.* 2002;7:S12–S13
41. Ritvo ER, Freeman BJ, Scheibel AB, et al. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLASNAC Autopsy Research Report. *Am J Psychiatry.* 1986;143:862–826
42. Bauman ML, Kemper TL. *The Neurobiology of Autism.* Baltimore, MD: The Johns Hopkins University Press; 1994
43. Bailey A, Luthert P, Dean A, et al. A clinicopathological study of autism. *Brain.* 1998;121:889–905
44. Black J. The puzzle of pink disease. *J Roy Soc Med.* 1999;92:478–481
45. Dally A. The rise and fall of pink disease. *Soc Soc Hist Med Bull.* 1997;10:291–304
46. Kondo K. Congenital Minamata disease: warnings from Japan's experience. *J Child Neurol.* 2000;15:458–464
47. Matsuishi T, Shiotsuki Y, Yoshimura K, Shoji H, Imuta F, Yamashita F. High prevalence of infantile autism in Kurume city, Japan. *J Child Neurol.* 1987;2:268–271
48. Tanoue Y, Oda S, Asano F, Kawashima K. Epidemiology of infantile autism in southern Ibaraki, Japan. *J Autism Dev Disord.* 1998;18:155–156
49. Sugiyama T, Abe T. The prevalence of autism in Nagoya, Japan: a total population study. *J Autism Dev Disord.* 1989;19:87–96
50. Kurita H. Clinical studies of pervasive developmental disorders in Japan. *Psychiatry Clin Neurosci.* 1996;50:165–170
51. Hoshino Y, Kumashiro H, Yashima Y, Tachibana R, Watanabe M. The epidemiological study of autism in Fukushima-ken. *Folia Psychiatr Neurol Jpn.* 1982;36:115–124
52. Institute of Medicine. *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders.* Stratton K, Gable A, McCormick MC, eds. Washington DC: National Academy Press; 2001
53. Bingham M, Copes R, Srour L. Exposure to thimerosal in vaccines used in Canadian infant immunization programs, with respect to risk of neurodevelopmental disorders. *Canadian Commmunicable Disease Report.* 2002;28:69–80
54. Myers GL, Davidson PW, Cox C, Shamlaye C, Cernichiari E, Clarkson TW. Twenty-seven years studying the human neurotoxicity of methylmercury exposure. *Environ Res.* 2000;83:275–285
55. Aschner M. Mercury toxicity. *J Pediatr.* 2001;138:450–451
56. Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol.* 1997;19:417–428
57. Grandjean P, Budtz-Jorgensen E, White RF, et al. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am J Epidemiol.* 1999;150:301–305
58. Myers GJ, Davidson PW, Cox C, et al. Summary of the Seychelles child development study on the relationship of fetal methylmercury exposure to neurodevelopment. *Neurotoxicology.* 1995;16:711–716
59. Myers GH, Davidson PW, Palumbo D, et al. Secondary analysis from the Seychelles Child Development Study: the Child Behavior Checklist. *Environ Res.* 2000;84:12–19
60. Noterdaeme M, Minow F, Amorosa H. Applicability of the Child Behavior Checklist in developmentally delayed children. *Z Kinder Jugendpsychiatr Psychother.* 1999;27:183–188

# Medical Information Systems in Pediatrics

The safety, effectiveness, impact, and risks of medical information systems have received little attention from clinical investigators in pediatrics. Krishna and colleagues'[1] study of the impact of a multimedia asthma education program published in this issue of *Pediatrics* is an exception to this observation and a wonderful example of a clinical research study on the effects of a medical information system.

Information systems that collect, process, and disseminate medical information are ubiquitous in our practice of pediatric medicine. These information resources serve a variety of functions, but they all have 1 thing in common: they are being used in a high-stakes environment. Technical glitches such as programming errors,[2] hardware malfunctions, communication failures, and data corruption or data loss can endanger the well-being of our patients. Human-machine interface errors like inappropriate use (a program designed for adults used in pediatrics), incomplete or inaccurate data entry, rearranged physician priorities, and the generation of false expectations and overreliance (the program will tell me when I made a mistake) all may lead to medical errors and subsequently to morbidity and mortality.

Despite their increasing presence, relatively little effort has been undertaken to systematically gather evidence on the safety and efficacy of medical information systems used with pediatric patients. Information systems used in pediatrics are fundamentally different from adult systems. They must handle weight-based dosing, different history components (such as development), monitor growth based on age, and if targeted for use by a child, must be designed to be child-friendly in language and graphics.

In April 2002, the Bush Administration decided to retain a 3-year-old rule that gives the Food and Drug Administration power to demand that pharmaceutical companies conduct targeted studies to learn about medication side effects and set proper doses for children.[3] Linked to an incentive program by Congress, this "pediatric rule" has generated evidence on particular pediatric risks as well as pediatric-specific metabolism.

Medical information systems are burdened with inherent danger in conjunction with pediatric-specific risks as well as significant expenses. In the best interest of our patients, pediatricians should lobby for an extension of the "pediatric rule" to information systems in pediatric settings. I applaud *Pediatrics* for providing a forum for evidence-based pediatric medical informatics.

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REFERENCES

1. Krishna S, Francisco BD, Balas EA, König P, Graff GR, Madsen RW. Internet-enabled interactive multimedia asthma education program: a randomized trial. *Pediatrics.* 2003;111:503–510
2. McDaniel JG. Improving system quality through software evaluation. *Comput Biol Med.* 2002;32:127–140
3. Marshall E. Pediatric drug trials: challenge to FDA's authority may end up giving it more. *Science.* 2002;296:820–821

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# Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study

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## Summary

**Background** Thiomersal is a preservative containing small amounts of ethylmercury that is used in routine vaccines for infants and children. The effect of vaccines containing thiomersal on concentrations of mercury in infants' blood has not been extensively assessed, and the metabolism of ethylmercury in infants is unknown. We aimed to measure concentrations of mercury in blood, urine, and stools of infants who received such vaccines.

**Methods** 40 full-term infants aged 6 months and younger were given vaccines that contained thiomersal (diptheria-tetanus-acellular pertussis vaccine, hepatitis B vaccine, and in some children *Haemophilus influenzae* type b vaccine). 21 control infants received thiomersal-free vaccines. We obtained samples of blood, urine, and stools 3–28 days after vaccination. Total mercury (organic and inorganic) in the samples was measured by cold vapour atomic absorption.

**Findings** Mean mercury doses in infants exposed to thiomersal were 45·6 μg (range 37·5–62·5) for 2-month-olds and 111·3 μg (range 87·5–175·0) for 6-month-olds. Blood mercury in thiomersal-exposed 2-month-olds ranged from less than 3·75 to 20·55 nmol/L (parts per billion); in 6-month-olds all values were lower than 7·50 nmol/L. Only one of 15 blood samples from controls contained quantifiable mercury. Concentrations of mercury were low in urine after vaccination but were high in stools of thiomersal-exposed 2-month-olds (mean 82 ng/g dry weight) and in 6-month-olds (mean 58 ng/g dry weight). Estimated blood half-life of ethylmercury was 7 days (95% CI 4–10 days).

**Interpretation** Administration of vaccines containing thiomersal does not seem to raise blood concentrations of mercury above safe values in infants. Ethylmercury seems to be eliminated from blood rapidly via the stools after parenteral administration of thiomersal in vaccines.


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*See Commentary page 1711*

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## Introduction

Thiomersal is a preservative used in vaccines routinely administered to infants and children. Its antimicrobial activity is due to small amounts of ethylmercury; the usual dose of paediatric vaccine contains 12·5–25 μg of mercury.[1-3] When vaccines containing thiomersal are administered in the recommended doses, allergic reactions have been rarely noted, but no other harmful effects have been reported.[4] Massive overdoses from inappropriate use of products containing thiomersal have resulted in toxic effects.[5-9]

Mercury occurs in three forms: the metallic element, inorganic salts, and organic compounds (eg, methylmercury, ethylmercury, and phenylmercury). The toxicity of mercury is complex and dependent on the form of mercury, route of entry, dosage, and age at exposure. Mercury is present in the environment in inorganic and organic forms, and everyone is exposed to small amounts.[10,11] The main route of environmental exposure to organic mercury is consumption of predatory fish, especially shark and swordfish. A 6-ounce can of tuna contains 2–127 μg (average 17 μg) of mercury.[12] Freshwater fish (eg, walleye, pike, muskie, and bass) can also contain high concentrations of mercury.

Most of the toxic effects of organic mercury compounds take place in the central nervous system, although the kidneys and immune system can also be affected.[10,11,13] Organic mercury readily crosses the blood-brain barrier, and fetuses are more sensitive to mercury exposure than are children or adults. Data about potential differences in toxicity between ethylmercury and methylmercury are few. Both are associated with neurotoxicity in high doses; in-utero poisoning with methylmercury causes problems that are similar to cerebral palsy. Findings about the effect of low-dose methylmercury exposure on neurodevelopment in infants are contradictory.[14,15] In-utero exposure could be related to subtle neurodevelopmental effects (eg, on attention, language, and memory) that can be detected by sophisticated neuropsychometric tests— although the conclusion is confounded by concomitant ingestion of polychlorinated biphenyls in the patients investigated.[14,15]

No toxic effects of low-dose exposure to thiomersal in children have been reported.[3] The effect of the small amounts of mercury contained in vaccines on concentrations of mercury in infants' blood has not been extensively assessed, and the metabolism of ethylmercury in infants is unknown. We aimed to assess concentrations of mercury in full-term infants after administration of routine vaccinations according to the schedule used in the USA, and to obtain additional information about the presence of mercury at other body sites including urine and stool. Samples of hair and breast milk were also obtained from some mothers of infants participating in the study.

## Methods

### *Study populations*

We studied two groups of full-term infants who differed in their history of exposure to vaccines containing thiomersal. Infants in the exposure group were recruited at the Elmwood Pediatric Group, a large paediatric practice in Rochester, NY, USA, where vaccinations with thiomersal preservative were routinely given. 20 infants aged 2 months and 20 aged 6 months were studied at this practice to obtain information about the range of total thiomersal exposures likely to take place during infancy. The control group consisted of 21 infants who did not receive vaccines containing thiomersal and were recruited from the National Naval Medical Center, Bethesda, MD. All the infants were recruited during routine well-child examination and vaccination visits by the investigators (between November, 1999 and October, 2000). Written informed consent was obtained from parents for all procedures.

### *Vaccines*

Vaccines containing thiomersal that were given to infants in the exposure group included Tripedia (diphtheria-tetanus-acellular pertussis vaccine; Aventis Pasteur, Swiftwater, PA; 0·01% thiomersal, 25 μg mercury per dose) Engerix (hepatitis B vaccine; GlaxoSmithKline, Rixensart, Belgium; 0·005% thiomersal, 12·5 μg mercury per dose), and in some children HibTITER (*Haemophilus influenzae* type b conjugate vaccine, Wyeth-Lederle, Pearl River, NY, USA; 0·01% thiomersal, 25 μg mercury per dose). Vaccines administered to the control group included Infanix (diptheria-tetanus-acellular pertussis vaccine; GlaxoSmithKline, Rixensart, Belgium), Recombivax HB (hepatitis B vaccine; Merck, West Point, PA, USA), and ActHIB (*Haemophilus influenzae* b conjugate vaccine, Aventis Pasteur, Swiftwater, PA, USA).

### *Procedures*

We obtained vaccination histories—including type of vaccine, manufacturer, lot number, and dates of administration—from the medical records. In the exposure group, we obtained samples of heparinised whole blood, stool, and urine, during a visit 3–28 days after vaccination. Blood and urine were kept at 4°C, and stools were frozen until assessment. Urine was sampled by use of a urine bag at the clinic, and stool was taken from a diaper (nappy) provided by the parent. Whole blood and urine were obtained from the control children. At both sites, we obtained at least 50 hairs from the mother by cutting at the base near the scalp in the occipital area, to assess potential transplacental exposure of infants to mercury. Additionally, several samples of breastmilk or formula were obtained from mothers of infants at Elmwood Pediatric Group, as well as stool samples from a few infants who were not exposed to thiomersal.

We measured total mercury in all samples (and inorganic mercury in stool samples) by cold vapour atomic absorption as previously described.[16,17] The limit of reliable quantitation in this assay ranged between 7·50 nmol/L and 2·50 nmol/L, dependant on sample volume.

### *Population pharmacokinetic calculations*

To estimate the half-life of thiomersal mercury in the blood, we developed a prediction model for the expected concentrations of mercury in blood for half-lives of mercury ranging from 1 day to 45 days, on the basis of bodyweight of the infant, the doses of thiomersal administered, and the times between the individual doses of thiomersal and when the blood was obtained. To do these calculations, we assumed that 5% of the mercury dose was distributed to blood,[7] that blood volume represented about 8% of the infant's bodyweight, and that elimination of mercury from blood followed a single-compartment model with first-order kinetics. For each possible half-life between 1 and 45 days, we then calculated the difference between the predicted and actual recorded concentrations in blood for each infant. Only measurements within the range of reliable quantitation were used in these calculations.

The best estimate of the blood half-life of mercury was judged to be the hypothetical half-life, which resulted in the smallest difference between predicted and observed values. We constructed a 95% CI based on a likelihood ratio for this estimate with the assumption that errors from the decay model were independent, additive, and normally distributed. The 95% confidence limits were the

| | Infants aged 2 months | | Infants aged 6 months | |
|---|---|---|---|---|
| | Thiomersal-exposed (n=20) | Controls (n=11) | Thiomersal-exposed (n=20) | Controls (n=10) |
| Bodyweight (kg) | | | | |
| Mean (range) | 5·3 (4·0–6·4) | NR | 8·1 (6·7–10·6) | NR |
| Total mercury exposure (μg)* | | | | |
| Mean (range) | 45·6 (37·5–62·5) | 0 | 111·3 (87·5–175·0) | 0 |
| Blood mercury (nmol/L) | | | | |
| Number of samples tested | 17 | 8 | 16 | 7 |
| Number with mercury in range | 12 | 1 | 9 | 0 |
| Mean (SD)† | 8·20 (4·85) | 4·90 | 5·15 (1·20) | .. |
| Median (IQR)† | 6·15 (4·60–10·85) | 4·90 | 5·30 (4·55–6·10) | .. |
| Range† | 4·50–20·55 | .. | 2·85–6·90 | .. |
| Urinary mercury (nmol/L) | | | | |
| Number of samples tested | 12 | 6 | 15 | 8 |
| Number with mercury in range | 1 | 0 | 3 | 0 |
| Mean (SD)† | 3·8‡ | .. | 5·75 (1·05) | .. |
| Median (range)† | 3·8‡ | .. | 6·2 (4·55–6·45) | .. |
| Stool mercury (ng/g dry weight) | | | | |
| Number of samples tested | 12 | NT | 10 | NT |
| Number with mercury in range | 12 | .. | 10 | .. |
| Mean (SD)† | 81·8 (40·3) | .. | 58·3 (21·2) | .. |
| Median (IQR)† | 83·5 (47·0–121·3) | .. | 58·0 (42·0–68·5) | .. |
| Range† | 23·0–141·0 | .. | 29·0–102·0 | .. |

NR=Not recorded. NT=not tested. *Via vaccination. †All calculations done only with samples within range of accurate quantitation. ‡Only one value so SD and range are not applicable.

**Concentrations of mercury in blood, urine, and stool of infants who received vaccines containing thiomersal and those who did not**

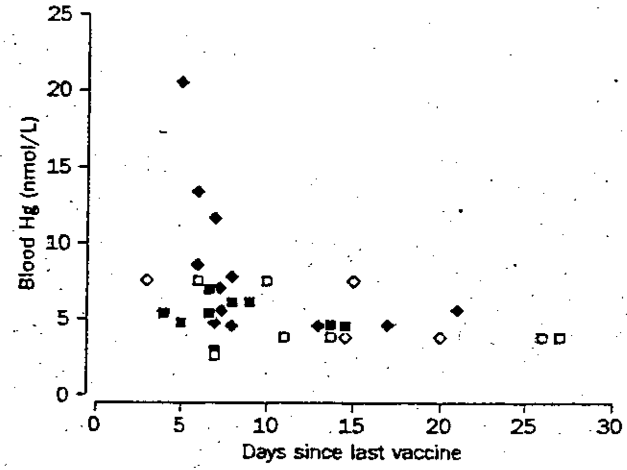


Figure 1: **Blood mercury concentrations in infants aged 2 months (diamonds) and 6 months (squares) by time of sampling**

Filled symbols represent measured values and open symbols represent samples at the limit of quantitation, either 7·50 nmol/L, 3·75 nmol/L, or 2·5 nmol/L, dependent on sample volume.

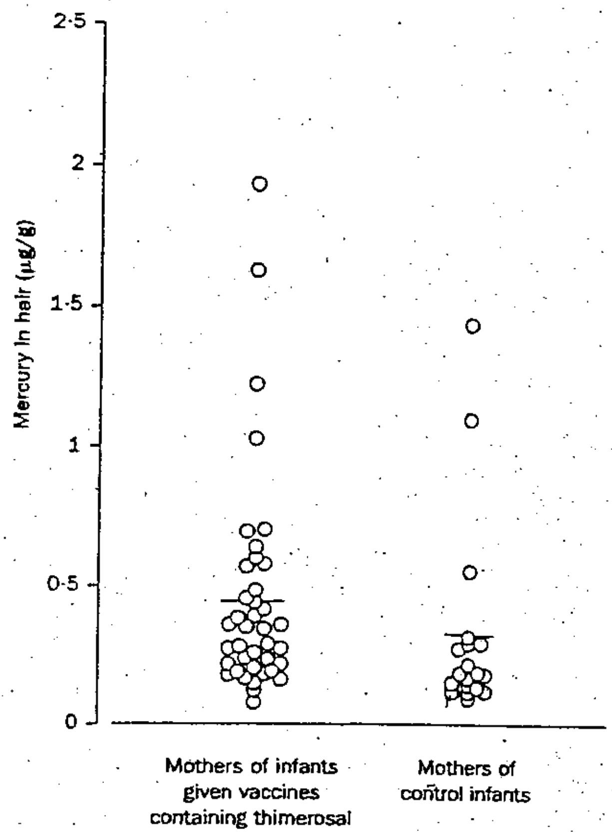


Figure 2: **Mercury concentrations in hair from mothers of infants**

Bar represents mean concentration of mercury in maternal hair.

points where the curve crossed the minimum sum of squares multiplied by $1+\chi^2(1)/(n-1)$ where n is the number of data points and $\chi^2(1)$ is the upper 5% point of the $\chi^2$ distribution on one degree of freedom.

*Statistical analysis*

Because this was a descriptive study we did no formal calculations for sample size. Student's *t* test and Fisher's exact test were used to compare results for the exposure and control group, with $p \leq 0.05$ judged to be significant.

*Role of the funding source*

The sponsors of the study approved the study design but had no other involvement in the in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

61 infants were enrolled in this study (table). Among infants aged 2 months in the exposure group, samples were taken from eight within 7 days of vaccination, from five between 8 and 14 days after vaccination, and from seven between 15 and 21 days after vaccination. Among 6-month-old infants in the exposure group, samples were taken from seven between 4 and 7 days after vaccination, from eight between 8 and 14 days after vaccination, and from five between 15 and 27 days after vaccination. Samples were obtained from infants in the control group at regularly scheduled visits at 2 or 6 months of age. All children remained healthy throughout the study and during 24–36 months of follow-up.

Sufficient volumes of blood (≥1 mL) for the measurement of mercury by the atomic absorption technique were obtained from 17 infants aged 2 months and 16 aged 6 months in the exposure group. Mercury concentrations were below the range of reliable quantitation in five of 17 blood samples from 2-month-olds, and seven of 16 blood samples from 6-month olds ($p=0.48$). The mean concentration of blood mercury in samples with quantifiable mercury was higher in 2-month-olds than in 6-month olds (difference 3·05 nmol/L, 95% CI 0·03–1·24, $p=0.06$), but was low in both these groups (table). Sufficient blood volumes for measurement of mercury were obtained from 15 infants in the control group, including eight aged 2 months and seven aged 6 months. Blood mercury was below the level of reliable quantitation in seven of the eight samples from the 2-month-olds and in all seven samples from 6-month-olds. The only detectable value from the control group was 4·65 nmol/L.

Overall, mercury concentrations were below the range of quantitation in 12 of 33 samples from thiomersal-exposed infants and in 14 of 15 unexposed infants ($p=0.04$). The highest level of blood mercury detected in any infant in this study was 20·55 nmol/L, which was measured 5 days after vaccination in a 2-month-old infant weighing 5·3 kg, who had received vaccines (Tripedia and Engerix B) containing a total dose of 37·5 µg mercury. The relation between time between vaccination and sampling and the concentration of mercury in the blood in the exposed group is shown in figure 1. Although mercury concentrations were uniformly low, the highest levels were recorded soon after vaccination.

Mercury was undetectable in most of the urine samples from the infants in this study. Only one of 12 urine samples from 2-month-olds, and three of 15 from 6-month-olds in the exposure group, and none of the 14 samples from the controls, contained detectable mercury. The highest concentration of urinary mercury detected was 6·45 nmol/L, in a 6-month old infant in the exposure group (table).

Stool samples were collected from infants in the exposure group. All of the stool samples from infants who received thiomersal-containing vaccines had detectable mercury, with concentrations in stools from 2-month-old infants slightly higher than those in 6-month-olds ($p=0.098$, table). As expected, most of the mercury in stools was inorganic. Stool samples were not obtained from control infants; therefore, to determine

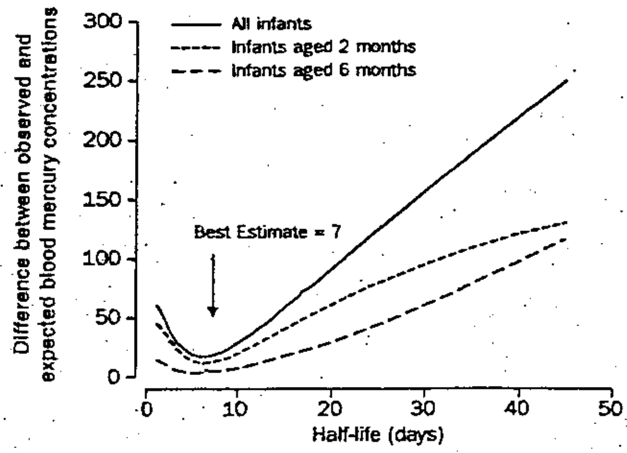


Figure 3: **Estimated blood half-life of mercury in infants who were exposed to thiomersal**

Lines represent sum of square of differences between observed concentrations of blood mercury (nmol/L) and those predicted for every individual infant on the basis of bodyweight and time of sampling, with a series of hypothetical half-lives shown on x axis. Arrow shows point with lowest value for squared difference, indicating best estimate for serum half-life.

whether dietary intake could contribute to the mercury content of stools, we also obtained samples from nine infants at Elmwood Pediatric Group who were age-matched with the infants in the exposure group and were not exposed to vaccines containing thiomersal. The mean mercury concentration in the stools of these infants was 22 ng/g dry weight (SD 16), which was significantly lower (p=0·002) than the mean of the samples collected from thiomersal-exposed infants.

Amounts of mercury measured in maternal hair are shown in figure 2. The mean concentration of hair mercury in mothers of the exposure group was 0·45 μg/g hair, whereas the mean amount in mothers of the control infants was 0·32 μg/g (p=0·22). Eight mothers of infants in the 6-month-old cohort provided breast milk samples. Concentrations of mercury in these samples were low (mean=0·30 μg/g, range 0·24–0·42 μg/g).

We estimated the half-life of mercury in blood after vaccination to be 7 days, since this result gave the smallest difference between the expected and recorded (measured) concentration (figure 3). The 95% CI around this estimate was 4–10 days. The half-life estimate was very similar when only measurements in 2-month-olds (7 days, 95% CI 4–11) or 6-month-olds (5 days, 3–9) were included, suggesting that the rate of elimination of thiomersal mercury from blood was similar in both age-groups.

## Discussion

We have shown that very low concentrations of blood mercury can be detected in infants aged 2–6 months who have been given vaccines containing thiomersal. However, no children had a concentration of blood mercury exceeding 29 nmol/L (parts per billion), which is the concentration thought to be safe in cord blood;[18] this value was set at ten times below the lower 95% CI limit of the minimal cord blood concentration associated with an increase in the prevalence of abnormal scores on cognitive function tests in children. Blood mercury concentrations indicate concentrations in organs well.[18]

Although our study was not designed as a formal assessment of the pharmacokinetics of mercury, we did obtain samples of blood at various time points after exposure. Assessment of these samples suggested that the blood half-life of ethylmercury in infants might differ from the 40–50 day half-life of methylmercury (range 20–70 days) in adults and breastfeeding infants.[18,19] The concentrations of blood mercury 2–3 weeks after vaccination noted in our study were not consistent with such a long half-life, but suggested a half-life of less than 10 days. However, this conclusion is based on several assumptions and a very simple model, and does not take into account the fact that at least some of the mercury detected in the blood of the infants in this study is likely to have been derived from exposures other than vaccination. Because of the short period between vaccination and sampling, the findings of Strajich and colleagues[20] could be consistent with either a 6-day or 40-day half-life, but are otherwise consistent with the assumptions made in our model. Because we expected a 45-day half-life on the basis of methylmercury pharmacokinetics, the first blood samples were obtained 3 days after vaccination. Blood samples taken in the first 72 hours after vaccination, stool samples obtained every 24 h, and samples from premature newborn babies (weighing ≥2000 g) given a birth dose of hepatitis B vaccine would have helped us to reach stronger conclusions. Thus, additional studies of the pharmacology of thiomersal in infants are underway.

At the times tested after vaccination, mercury excretion in urine in our study population was low. By contrast, concentrations of mercury in stool were high, and combined with the finding that stool mercury concentrations in infants who were not exposed to thiomersal were significantly lower is consistent with the hypothesis that the gastrointestinal tract represents a possible mode of elimination of thiomersal mercury in infants.

Overall, the results of this study show that amounts of mercury in the blood of infants receiving vaccines formulated with thiomersal are well below concentrations potentially associated with toxic effects. Coupled with 60 years of experience with administration of thiomersal-containing vaccines, we conclude that the thiomersal in routine vaccines poses very little risk to full-term infants, but that thiomersal-containing vaccines should not be administered at birth to very low birthweight premature infants. Decisions about the elimination of thiomersal from these vaccines must balance the potential benefit of reduced exposure to mercury against the risks of decreased vaccine coverage because of higher costs, the risk of sepsis in recipients because of bacterial contamination of preservative-free formulations, and the risks of exposure to alternative preservatives that might replace thiomersal.

*Conflict of interest statement*

None declared.

*Contributors*

M Pichichero and J Treanor contributed to the study conception and design; obtained, assessed, and interpreted data; drafted and revised the manuscript; and provided statistical expertise and supervision. E Cernichiari contributed to analysis and interpretation of data, revision of the manuscript, and technical support. J Lopreiato contributed to revision of the manuscript, and obtained data.

*Acknowledgments*

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**References**

1 Clements CJ, Ball LK, Ball R, Pratt D. Thiomersal in vaccines. *Lancet* 2000; 355: 1279–80.

2 American Academy of Pediatrics, Committee on Infectious Diseases, and Committee on Environmental Health. Thimerosal in vaccines—An interim report to clinicians. *Pediatrics* 1999; 104: 570–74.

3 Ball LK, Ball R, Pratt, RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics* 2001; 107: 1147–54.

4 Cox NH, Forsyth A. Thimerosal allergy and vaccination reactions. *Contact Dermatitis* 1988; 18: 229–33.

5 Axton JH. Six cases of poisoning after a parenteral organic mercurial compound (merthiolate). *Postgrad Med J* 1972; 561: 417–21.

6 Fagan DG, Pritchard JS, Clarkson TW, Greenwood MR. Organ mercury levels in infants with omphaloceles treated with organic mercurial antiseptic. *Arch Dis Child* 1977; 52: 962–64.

7 Matheson DS, Clarkson TW, Gelfand EW. Mercury toxicity (acrodynia) induced by long-term injection of gammaglobulin. *J Pediatr* 1980; 97: 153–55.

8 Lowell JA, Burgess S, Shenoy S, Curci JA, Peters M, Howard TK. Mercury poisoning associated with high-dose hepatitis-B immune globulin administration after liver transplantation for chronic hepatitis B. *Liver Transpl Surg* 1996; 2: 475–78.

9 Pfab R, Muckter H, Roider G, Zilker T. Clinical course of severe poisoning with thimerosal. *J Toxicol Clin Toxicol* 1996; 34: 453–60.

10 Clarkson TW. Mercury: major issues in environmental health. *Environ Health Perspect* 1992; 100: 31–38.

11 Clarkson TW. The toxicology of mercury. *Crit Rev Clin Lab Sci* 1977; 34: 369–403.

12 Yess NJ. US food and Drug Administration survey of methylmercury in canned tuna. *J AOAC Int* 1993; 76: 36–38.

13 Shenker BJ, Guo TL, Shapiro IM. Low-level methylmercury exposure causes human T-cells to undergo apoptosis: evidence of mitochondrial dysfunction. *Environ Res* 1998; 77: 149–59.

14 Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; 6: 417–28.

15 Davidson PW, Myers GJ, Cox C, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. *JAMA* 1998; 280: 701–07.

16 Cernichiari E, Toribara TY, Liang L, et al. The biological monitoring of mercury in the Seychelles study. *Neurotoxicology* 1995; 16: 613–28.

17 Cernichiari E, Brewer R, Myers GJ, et al. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. *Neurotoxicology* 1995; 16: 705–10.

18 Nielsen JB, Andersen O, Grandjean P. Evaluation of mercury in hair, blood and muscle as biomarkers for methylmercury exposure in male and female mice. *Arch Toxicol* 1994; 68: 317–21.

19 National Academy of Sciences. Toxicologic effects of methylmercury. Washington DC: National Research Council, 2000.

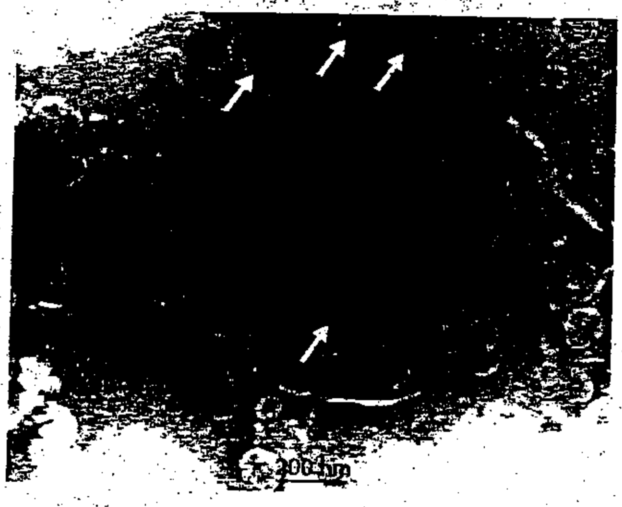
20 Stajich GV, Lopez GP, Harry SW, Sexson WR. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. *J Pediatr* 2000; 136: 679–81.

# Clinical picture

## Fatal meningococcal septicaemia with "blebbing" meningococcus

*Ellen Namork, Petter Brandtzaeg*

A 20-year-old previously healthy man was found unconscious in bed early in the morning. He was febrile and had an extensive haemorrhagic and cyanotic rash. He developed severe septic shock, multiple organ failure, and died 6 h later. *Neisseria meningitidis* serogroup B grew in one blood culture. Electron-microscopy of his plasma showed meningococci releasing many outer membrane vesicles ("blebs") known to harbour endotoxin (lipopolysaccharide). The endotoxin level in his plasma was 1700 endotoxin units/mL, which is equal to the activity of 170 ng/mL of purified lipopolysaccharide from *Escherichia coli*. Release of "blebs" (figure; magnified ×65 000, arrows) from rapidly growing meningococci is thought to contribute to the very high levels of endotoxin in plasma which characterise fatal meningococcal septicaemia.

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## STATEMENT FROM THE COMMITTEE ON SAFETY OF MEDICINES

## FURTHER DATA SUPPORT SAFETY OF THIOMERSAL IN VACCINES

On 12 February 2003, the Committee on Safety of Medicines (CSM) considered further evidence which supports the safety of thiomersal (which contains ethylmercury) in vaccines.

Two independently-conducted UK epidemiological studies that investigated the safety of thiomersal-containing vaccines for infants have been recently completed. These studies showed no evidence of adverse developmental effects from exposure to levels of thiomersal at the amounts used in existing UK vaccines. A further study in infants has shown that ethylmercury is rapidly excreted from the body following administration of thiomersal-containing vaccines.

The CSM Chairman, Professor Alasdair Breckenridge, said 'These new studies reinforce CSM advice from 2001 that there is no evidence of neurological adverse effects caused by thiomersal in vaccines according to the routine UK immunisation schedule. The balance of benefits and risks of thiomersal-containing vaccines therefore remains overwhelmingly positive'.

**Key points**

- The only vaccines used in the UK routine immunisation programme that contain thiomersal are the diphtheria, tetanus and wholecell pertussis (DTwP) and diphtheria and tetanus vaccines. Thiomersal is also present in some influenza and hepatitis B vaccines. There is no thiomersal in MMR, Hib, oral polio, meningitis C or BCG vaccine

- No harmful effects are known to be associated with thiomersal at amounts used in vaccines, except for minor allergic reactions such as redness and swelling at the injection site

- Two new UK epidemiological studies, involving more than 100,000 children, further support the CSM position. Another new study has shown that ethylmercury from vaccines does not build up in the body and is rapidly excreted.

- The World Health Organisation has also recently concluded that there is no evidence of toxicity in infants, children or adults exposed to thiomersal in vaccines

- There is a worldwide goal to reduce environmental mercury exposure from avoidable sources in general. Use of thiomersal in vaccines has not been banned but US and European regulators recommended in 1999 that reducing the use of thiomersal in vaccines will contribute to this goal. CSM endorsed this recommendation in 1999 and continues to do so. Several UK licensed vaccines have had thiomersal removed or levels of thiomersal reduced since 1999.

**What is thiomersal and why is it used in vaccines?**

Thiomersal is an ethylmercury-containing compound that has played an important role either as a preservative or in the initial stages of the manufacture of some vaccines for over 60 years.

**What have the concerns been about?**

The mercury content of thiomersal has led to concerns that it may affect brain development when given in vaccines. However, no harm to brain development has been demonstrated as a result of the very small amounts of thiomersal present in some vaccines.

**What is the view of the Committee on Safety of Medicines (CSM)?**

The CSM has kept the issue of thiomersal in vaccines under review. In September 2001, the CSM reviewed the available data relating to the safety of thiomersal in vaccines and advised that there is no evidence of neurological adverse effects caused by levels of thiomersal in vaccines. The only evidence of harm due to thiomersal was a small risk of hypersensitivity reactions (that typically include skin rashes or local swelling at the site of injection). The CSM concluded that the balance of risks and benefits of thiomersal-containing vaccines remains overwhelmingly positive.

**What is the latest evidence considered by CSM?**

The two new epidemiological studies specifically set out to assess the safety of thiomersal in vaccines used according to the UK childhood immunisation schedule. One study, funded jointly by the World Health Organisation and the Public Health Laboratory Service, was conducted using the UK's General Practice Research Database (GPRD) which holds data on health care for 3 million patients (~5% of the UK population). The second study was funded by the Department of Health and used the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC).

These studies investigated whether there is any link between early thiomersal exposure through immunisation and developmental and behavioural disorders, including autism, in more than 100,000 children in total. Both studies produced very reassuring results in that neither supports an association between thiomersal exposure through the UK immunisation programme and neurodevelopmental disorders in children.

A further study, published in The Lancet in November 2002, looked at how a small child's body breaks down ethylmercury from vaccines. This study found that ethylmercury from vaccines does not build up in the body and is rapidly excreted.

**What does the new evidence tell us?**

On 12 February 2003, the CSM carefully reviewed these 3 studies and considered that they provide very reassuring evidence on the safety of the thiomersal in vaccines and further support its advice of 2001.

**What is the view of the World Health Organisation (WHO)?**

The World Health Organisation's Global Advisory Committee on Vaccine Safety (GACVS) has also kept this issue under review and concluded in November 2002 that there is no evidence of toxicity in infants, children or adults exposed to thiomersal in vaccines.

**What is the position in the Unites States of America and Europe with regard to use of thiomersal in vaccines?**

Use of thiomersal in vaccines has not been banned in USA and Europe and there is strong evidence to show that thiomersal in vaccines does not cause neurological adverse effects. Despite this, as part of a global goal to reduce exposure to mercury from avoidable sources in general, European and American regulators recommended in 1999 that vaccine manufacturers phase out use of thiomersal wherever possible. CSM endorsed this recommendation in 1999 and continues to do so.

**What is being done in the UK to remove thiomersal from vaccines?**

Several UK licensed vaccines have had thiomersal removed or levels of thiomersal reduced since 1999. Manufacturers are actively developing research programmes to eliminate, substitute or reduce thiomersal in vaccines in accordance with the European recommendations. This may take time because manufacturers are required to ensure that the replacement or elimination of thiomersal does not affect the safety or efficacy of the final vaccine and there is no set timeframe to phase out the use of thiomersal in vaccines. While these changes are underway, the current view is that there is no reason to change current immunisation practices with thiomersal-containing vaccines.

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# Autism and Thimerosal-Containing Vaccines

## Lack of Consistent Evidence for an Association

Paul Stehr-Green, DrPH, MPH, Peet Tull, Michael Stellfeld, MD, Preben-Bo Mortenson, DrMedSC, Diane Simpson, MD, PhD

**Background:** In 1999, concerns were raised that vaccines containing the preservative Thimerosal™ might increase the risk of autism and/or other neurodevelopmental disorders.

**Methods:** Between the mid-1980s through the late-1990s, we compared the prevalence/incidence of autism in California, Sweden, and Denmark with average exposures to Thimerosal-containing vaccines. Graphic ecologic analyses were used to examine population-based data from the United States (national immunization coverage surveys and counts of children diagnosed with autism-like disorders seeking special education services in California); Sweden (national inpatient data on autism cases, national vaccination coverage levels, and information on use of all vaccines and vaccine-specific amounts of Thimerosal); and Denmark (national registry of inpatient/outpatient-diagnosed autism cases, national vaccination coverage levels, and information on use of all vaccines and vaccine-specific amounts of Thimerosal).

**Results:** In all three countries, the incidence and prevalence of autism-like disorders began to rise in the 1985–1989 period, and the rate of increase accelerated in the early 1990s. However, in contrast to the situation in the United States, where the average Thimerosal dose from vaccines increased throughout the 1990s, Thimerosal exposures from vaccines in both Sweden and Denmark—already low throughout the 1970s and 1980s—began to decrease in the late 1980s and were eliminated in the early 1990s.

**Conclusions:** The body of existing data, including the ecologic data presented herein, is not consistent with the hypothesis that increased exposure to Thimerosal-containing vaccines is responsible for the apparent increase in the rates of autism in young children being observed worldwide.
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## Introduction

In June of 1999, concerns were raised that children vaccinated with products containing the preservative Thimerosal™ could receive doses of organic mercury (specifically, the thiosalicylate salt of ethylmercury) that exceeded existing guidelines for intake of methylmercury.[1] These concerns were based on extrapolations from the known effects of prenatal methylmercury exposure.[2] Because there are limited data on the toxicology and pharmacokinetics of Thimerosal and ethylmercury, for the purpose of these extrapolations it was assumed that many features of the toxicity of ethylmercury were qualitatively similar to those of methylmercury.[1]

It was subsequently suggested that the apparent increase in the incidence of autism in the United States in the 1990s occurred at about the same time that *Haemophilus influenzae* b (Hib) and hepatitis B (hep B) vaccines were first universally recommended (i.e., in 1990 and 1991, respectively), thereby increasing the average cumulative exposure to Thimerosal from vaccines administered to infants. Prior to that time, the only sources of Thimerosal from vaccines on the recommended childhood immunization schedule were diphtheria–tetanus–pertussis (DTP) (later replaced by diphtheria–tetanus–acellular pertussis [DTaP]) and diphtheria–tetanus (DT) vaccines. Although the maximum theoretical dose of Thimerosal from vaccines varied depending on the brand and combination vaccines used, most children in the United States who received the four universally recommended doses of

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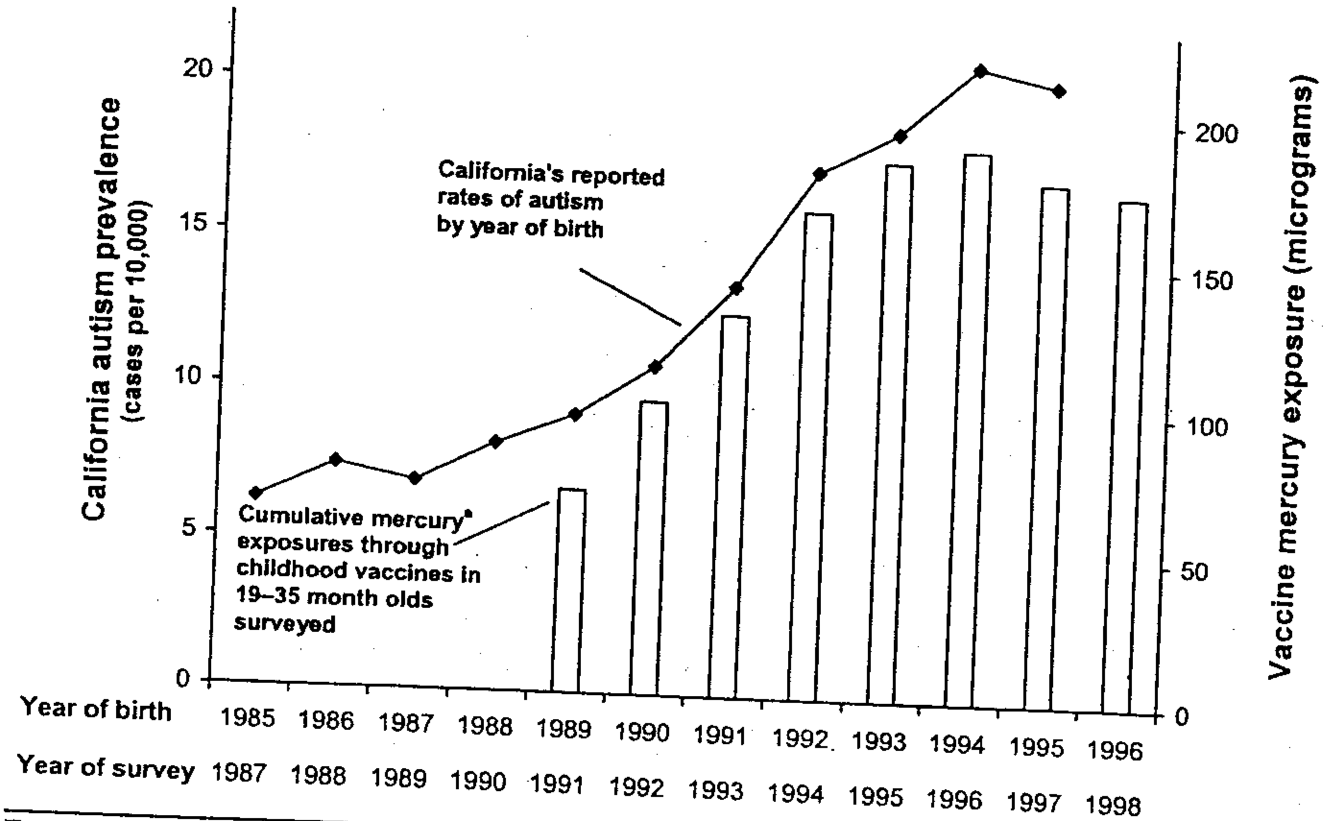


**Figure 1.** Graphical ecologic analysis presented by Blaxill[3] to the Institute of Medicine on July 16, 2001, comparing the estimated average cumulative dose of mercury exposure in the United States from vaccines, and the estimated prevalence (per 10,000 population) of children diagnosed with autism-like disorders seeking special education services for autism in California from 1987 to 1998, by birth-year cohort.
[a]Includes DPT, *Haemophilus influenza* B, and hepatitis B exposures weighted by survey year compliance.

DTaP/DTP/DT, four doses of Hib, and three doses of hep B in 1999 would have received a 237.5 μg cumulative dose of ethylmercury by age 2 years.

In July 2001, the Institute of Medicine (IOM) Immunization Safety Review Committee held a public meeting to review data and testimony regarding the alleged association of neurodevelopmental effects (including autism) and Thimerosal-containing vaccines. At this meeting, Blaxill[3] presented an ecologic analysis comparing the estimated average cumulative dose of mercury exposure (i.e., the average ethylmercury dose, calculated by multiplying the amount of Thimerosal in the various vaccines by the vaccine-specific coverage rate for U.S. children aged 19 to 35 months, by birth year cohort) to the estimated prevalence of autism in children in California per 10,000 population, by birth year. The prevalence of autism was defined as occurrence of persons with autism or other pervasive developmental disorders (PDD), based on an individualized client development evaluation performed at intake into the California Department of Developmental Services regional and developmental center system during 1987–1998 and coded as International Classification of Diseases (ICD)-9 codes 299.1, 299.80, or 299.88[4]; or (1) "Autism, full syndrome" (no ICD-9 code specified); (2) "Autism, residual state" (no ICD-9 code specified); or (3) "Autism suspected, not diagnosed" (no ICD-9 code specified).[5] The graphical presentation of these data (Figure 1) showed that the number of children in California coded as having autism-like disorders seeking special education services per 10,000 population remained reasonably constant through the mid-1980s, began to rise slightly in 1988, and then began to rise more dramatically in 1990.

As with most ecologic analyses, these data had several limitations. Nonetheless, because of the high level of public interest and the potentially important public health implications, collection of additional ecologic data to further examine this alleged association was performed. In conducting this investigation, we consulted with public health officials and researchers in Sweden and Denmark; both countries have historically maintained high-quality records on vaccine components, recommended vaccination schedules, population vaccination coverage rates, and the occurrence of autism-like disorders.

## Methods

In Sweden, data were collected at the national level on cases of autism (defined as "infantile autism, including atypical autism" [ICD-9 codes 299.x for 1987–1997 and ICD-10 codes F84.x for 1997–1999]) diagnosed in inpatient settings among 2 to 10 year olds during from 1987 to 1999. Data collection also included vaccination coverage levels dating back to 1980 as well as administrative information from the Swedish Institute for Infectious Disease Control for the time period(s) of use and vaccine-specific amounts of Thimerosal for all vaccines used in Sweden.

For each birth-year cohort, the average cumulative dose of ethylmercury from vaccines was estimated by multiplying the amount of ethylmercury in Thimerosal-containing vaccines used in Sweden by the vaccine-specific coverage rate for Swedish children aged <2 years. The incidence rate of autism was calculated by dividing the number of cases of autism diagnosed among 2- to 10-year-old inpatients during 1987–1999 by the total number of person-years accumulated during that time period for each annual cohort of children born between 1980 and 1996 (multiplied by 100,000 person-years). Using these data, the ecologic association of the birth-year, cohort-specific administration of Thimerosal-containing vaccines, and the incidence of autism requiring hospitalization among children born in Sweden from 1980 to 1996 was examined.

In Denmark, we examined data on incident cases of autism diagnosed in both inpatient and outpatient settings. The data were from a national registry of children with neurological disorders and compiled by researchers at the Danish National Centre for Register-Based Research. This registry included children who had been admitted to a psychiatric hospital or received outpatient care prior to 1994 with a diagnosis of "psychosis proto-infantilis" (ICD-8 code 299.00); "psychosis infantilis posterior" (ICD-8 code 299.01); or, from 1994 onward, "infantile autism" (ICD-10 code F84.0) or "atypical autism" (ICD-10 code F84.1). Data were also collected at the national level on vaccination coverage levels dating back to 1981, in addition to administrative information from the Danish Statens Serum Institut for the time period(s) of use and vaccine-specific amounts of Thimerosal for all vaccines used in Denmark.

The average cumulative dose of ethylmercury from vaccines for each birth-year cohort was estimated by multiplying the amount of ethylmercury in Thimerosal-containing vaccines used in Denmark by the vaccine-specific coverage rate for Danish children aged <10 months. The number of autism cases diagnosed among 2 to 10 year olds was totaled for each year between 1983 and 2000. Using these data, the ecologic association of the birth-year cohort-specific administration of Thimerosal-containing vaccines and the annual number of cases of autism diagnosed between 1983 and 2000 among children aged 2 to 10 years in Denmark was examined.

## Results

As shown in Figure 2, the incidence of autism diagnosed among Swedish inpatients aged 2 to 10 years old began to increase in the mid to late 1980s, rising from a rate of 5 to 6 inpatient-diagnosed cases per 100,000 person-years before 1985 to a peak rate of 9.2/100,000 in 1993. This was generally similar to the above-described trend in California during the same time period. Vaccination coverage has remained high in Sweden (i.e., almost always >95% for all age-specific antigens) since 1980, but the use of Thimerosal in vaccines in Sweden decreased and eventually disappeared by 1993. In fact, few vaccines containing Thimerosal were ever used throughout the history of childhood vaccination programs in Sweden. The major exception was the use of Thimerosal-containing DTP (used until 1979) and DT vaccines (used until 1992), both of which contained Thimerosal at a concentration of 0.01% (i.e., identical to the amount of Thimerosal contained in DTP and DT vaccines used in the United States). A very small number of children also received Thimerosal-containing single-antigen Hib vaccine and/or acellular pertussis vaccines used in a clinical trial prior to 1992. However, since 1992, Thimerosal has not been used in vaccines administered as part of the routine childhood vaccination program in Sweden, except for the very small number of children born to high-risk mothers (<1% of the annual birth cohort) who may have received Thimerosal-containing hep B. Thus, most children in Sweden who received the three recommended doses of Thimerosal-containing DTP/DT prior to 1992 would have received a 75-$\mu$g cumulative dose of ethylmercury by age 2 years.

As shown in Figure 3, the experience in Denmark was similar to that in Sweden, where the annual number of autism cases rose from <10 cases among 2 to 10 year olds before 1990 to a peak of 181 cases in 1999. This increase, which began around 1990, affected all age groups aged >2 years and resulted in an estimated prevalence of 8.1 cases per 10,000 persons at the end of 2000.[6] As in Sweden, vaccination coverage in Denmark has remained high (i.e., almost always ≥90% for all age-specific antigens) since 1980. In Denmark, throughout the period between 1970 and 1989, Thimerosal was used only in whole-cell pertussis (wP)-containing vaccines at a concentration of 0.01% (i.e., identical to the amount of Thimerosal in DT and pertussis-containing vaccines in the United States and Sweden). Therefore, children in Denmark who received the three recommended doses of Thimerosal-containing wP between 1970 and 1991 would have received a 125-$\mu$g cumulative dose of ethylmercury by age 10 months. In April 1992, the last batch of Thimerosal-containing wP vaccine was produced in Denmark, and its use was eliminated entirely by the end of 1992. Consequently, the proportion of children who received a 125-$\mu$g cumulative dose of ethylmercury by age 10 months decreased dramatically between 1991 and 1993. Thus, the apparent rise in diagnosed autism cases in Denmark, as in Sweden, occurred during a time of decreasing use (and eventual elimination) of Thimerosal-containing vaccines.

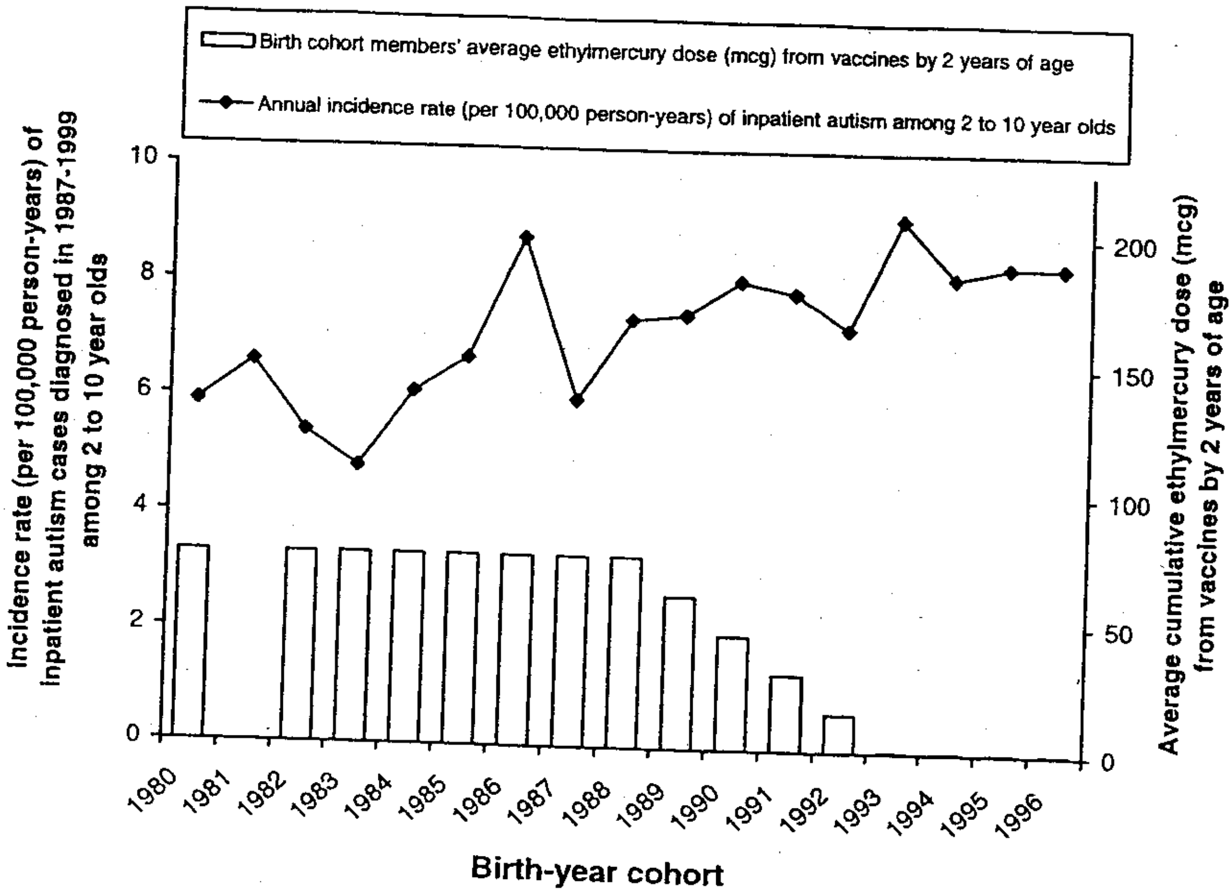


**Figure 2.** Graphical ecologic analysis comparing average cumulative ethylmercury dose received from vaccines and the incidence rate (per 100,000 person-years) of autism cases in children aged 2 to 10 years diagnosed during 1987–1999 in inpatient settings in Sweden, by birth-year cohort from 1980 to 1996. (Data not available for year 1981.)

## Discussion

At first glance, since the increasing vaccination coverage levels in the United States in the early 1990s likely reflect an increasing average exposure to Thimerosal from those vaccines, the results of the ecologic analysis presented to the IOM in July 2001, which showed proximate increases in autism incidence in California, could be argued to be generally consistent with the existence of an etiologic association. On closer examination, however, the upward trend in the prevalence of autism in California (and elsewhere in the United States) appears to have started, albeit at a more moderate rate, in the late 1980s—before the increase in vaccination coverage rates and/or the introduction of additional Thimerosal-containing vaccines (i.e., Hib and hep B) in the early 1990s. Similarly, the rate of autism in Sweden also appears to have began to increase in the mid to late 1980s and, in fact, may have started much earlier. Population-based data representative of the city of Gothenburg (Sweden's second-largest city) show an earlier increase in the prevalence of autism and autism-like conditions (excluding Asperger syndrome) from 4.0/10,000 children in 1980, to 7.5/10,000 in 1984, to 11.6/10,000 in 1988.[7–9]

Although the data from California are the most complete data currently available in the United States, the case definition used by the California Department of Developmental Services (described above) is somewhat vague and, therefore, difficult to verify and/or replicate. Furthermore, these data are likely subject to potential biases. For instance, at least some of the increase of reported cases of autism in California may have been stimulated by the growing availability of special education services for affected children during this time period. And even though the data systems in Sweden and Denmark achieve a remarkable level of validity and accuracy, similar confounding influences or biases may be present. For instance, several external events in Denmark, summarized below, may have spuriously increased the apparent number of autism cases.

- Prior to 1992, the data in the national register did not include cases diagnosed in one large clinic in Copenhagen (which accounts for approximately 20% of cases occurring nationwide).
- Prior to 1995, the autism cases reported to the national register reflected only cases diagnosed in inpatient settings.

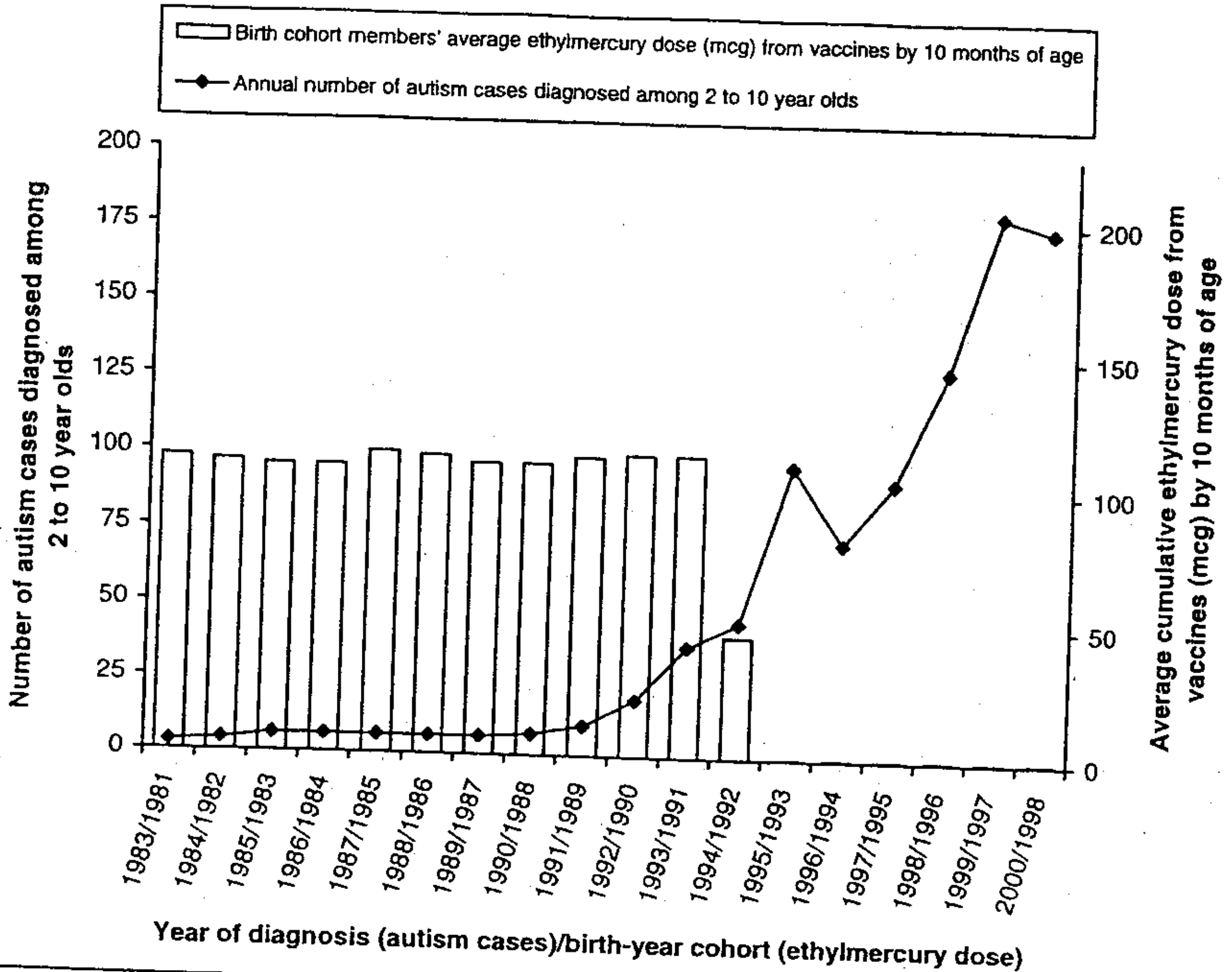


**Figure 3.** Graphical ecologic analysis comparing the average cumulative ethylmercury dose received from vaccines by birth-year cohort from 1981 to 1998, and the annual number of incident cases of autism in children aged 2 to 10 years diagnosed in Denmark from 1983 to 2000.

- In 1993, when Denmark switched from coding health outcomes using ICD-8 codes to ICD-10 codes, nationwide training seminars for clinicians on the new coding scheme may have stimulated reporting of autism cases (as well as other health outcomes).

Similarly, the data examined from Sweden reflected only cases diagnosed in inpatient settings, for which the data are readily available. Thus, changes over time in the rates of diagnosis of autism-like disorders in inpatient versus outpatient settings may have affected the ascertainment of cases, and differences in the distribution of the setting in which diagnoses have occurred may have affected the comparability of these results over time and among these three countries.

The apparent increase in diagnosed cases of autism may also be due, at least in part, to changes that have occurred over time in diagnostic criteria and increasing professional and public awareness of autism and related disorders. In fact, the diagnostic criteria for Asperger syndrome, Rett syndrome, and childhood disintegrative disorder were introduced for the first time in 1994 as subcategories of PDD.[4] Of note, these subcategories of PDD accounted for the largest increases in the reported California cases reflected in the data used in the ecologic analysis presented to the IOM.

Finally, at least some of the apparent discrepancy between the California findings and those in Sweden and Denmark are likely the result of the well-known shortcomings of ecologic data rather than a reflection of actual differences in the etiologic process of autism in these respective countries. Ecologic analyses, such as those presented herein, represent empirical investigations involving groups (as opposed to individual persons) as the unit of analysis. Such studies can be useful in exploring possible associations, as well as in searching for areas of possible further study, and are relatively easy to do since group-level data are often more readily available. However, the greatest difficulty in interpreting ecologic studies is that of adequately controlling confounding factors due to unavailability of data and/or methodologic limitations.[10] Given the ecologic nature of the analyses presented herein and the lack of available detailed data, we were unable to investigate other aspects of this alleged association (e.g., the specific timing of exposure and/or the onset of autism, the existence/nature of a lag time between exposure and disease onset, or the role of genetic predisposition or other co-factors) or the potential influence of confounding factors.

Nonetheless, even though the observed rise in autism cases in both Sweden and Denmark during a time of decreasing use (and eventual elimination) of Thimerosal-containing vaccines in the early 1990s was based on ecologic evidence (and is, therefore, subject to the aforementioned limitations), these results provide compelling evidence in sharp contrast to the alleged association observed in California, during the same time period, which was based on similar ecologic data. More robust studies are currently being planned at the Centers for Disease Control and Prevention and elsewhere to examine the possible association of Thimerosal-containing vaccines and neurodevelopmental problems (including autism) that will be designed to eliminate (or at least mitigate) these limitations (W.C. Thompson, National Immunization Program, Centers for Disease Control and Prevention, personal communication, 2002).

## Conclusion

After considering all the existing evidence, in September 2001 the IOM concluded that "the evidence is inadequate to accept or reject a causal relationship between exposure to Thimerosal from vaccines and . . . autism . . . . [However,]. . .the hypothesis is biologically plausible."[11] The authors of the IOM study found no consistent ecologic evidence linking the administration of Thimerosal-containing vaccines with an increasing incidence/prevalence of autism cases. Therefore, it is reasonable to conclude that the body of existing data, including the ecologic data presented herein, are not consistent with the hypothesis that increased exposure to Thimerosal-containing vaccines are responsible for the apparent increases in the rates of autism in young children being observed worldwide. Rather, it seems more plausible that other factors are affecting these changes, such as those mentioned above: an increased recognition of the disorder in the most and least developmentally delayed children (i.e., compared to children with IQs in the 50 to 70 range) and/or possibly other as-yet-unidentified environmental or genetic factors.

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## References

1. Ball LK, Ball R, Pratt RD. An assessment of Thimerosal use in childhood vaccines. Pediatrics 2001;107:1147–54.
2. Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 1997;19:417–28.
3. Blaxill M. Rising incidence of autism: association with Thimerosal. In: Institute of Medicine. Thimerosal-containing vaccines and neurodevelopmental disorders. Washington DC: National Academy Press, 2001:49–50.
4. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington DC: American Psychiatric Association, 1994.
5. California Department of Developmental Services. Changes in the population of persons with autism and pervasive developmental disorders in California's Developmental Services System: 1987 through 1998. Sacramento CA: California Health and Human Services Agency, 1999.
6. Madsen KM, Lauritsen MB, Pedersen CB, et al. Thimerosal and the occurrence of autism: negative ecological evidence from Danish population-based data. Pediatrics 2003. In press.
7. Gillberg C, Steffenburg S, Schaumann H. Is autism more common now than ten years ago? Br J Psychiatry 1991;158:403–9.
8. Gillberg C. Infantile autism and other childhood psychoses in a Swedish urban region. Epidemiologic aspects. J Child Psychol Psychiatry 1984;25:35–43.
9. Nordin V, Gillberg C. Autism spectrum disorders in children with physical or mental disability or both. I: clinical and epidemiological aspects. Dev Med Child Neurol 1996;38:297–313.
10. Morgenstern H. Uses of ecological analyses in epidemiologic research. Am J Public Health 1982;72:1336–44.
11. Institute of Medicine. Thimerosal-containing vaccines and neurodevelopmental disorders. Washington DC: National Academy Press, 2001.

# Safety of Thimerosal-Containing Vaccines: A Two-Phased Study of Computerized Health Maintenance Organization Databases

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ABSTRACT. *Objective.* To assess the possible toxicity of thimerosal-containing vaccines (TCVs) among infants.

*Methods.* A 2-phased retrospective cohort study was conducted using computerized health maintenance organization (HMO) databases. Phase I screened for associations between neurodevelopmental disorders and thimerosal exposure among 124 170 infants who were born during 1992 to 1999 at 2 HMOs (A and B). In phase II, the most common disorders associated with exposure in phase I were reevaluated among 16 717 children who were born during 1991 to 1997 in another HMO (C). Relative risks for neurodevelopmental disorders were calculated per increase of 12.5 μg of estimated cumulative mercury exposure from TCVs in the first, third, and seventh months of life.

*Results.* In phase I at HMO A, cumulative exposure at 3 months resulted in a significant positive association with tics (relative risk [RR]: 1.89; 95% confidence interval [CI]: 1.05–3.38). At HMO B, increased risks of language delay were found for cumulative exposure at 3 months (RR: 1.13; 95% CI: 1.01–1.27) and 7 months (RR: 1.07; 95% CI: 1.01–1.13). In phase II at HMO C, no significant associations were found. In no analyses were significant increased risks found for autism or attention-deficit disorder.

*Conclusions.* No consistent significant associations were found between TCVs and neurodevelopmental outcomes. Conflicting results were found at different HMOs for certain outcomes. For resolving the conflicting findings, studies with uniform neurodevelopmental assessments of children with a range of cumulative thimerosal exposures are needed. *Pediatrics* 2003;112:1039–1048; *cohort study, computerized medical record systems, language development disorders, speech disorders, thimerosal, vaccines.*

ABBREVIATIONS. Hg, mercury; EPA, Environmental Protection Agency; TCV, thimerosal-containing vaccine; HMO, health maintenance organization; VSD, Vaccine Safety Datalink; CDC, Centers for Disease Control and Prevention; LBW, low birth weight; ICD-9-CM, *International Classification of Diseases, Ninth Revision, Clinical Modification*; ADD, attention-deficit disorder; RR, relative risk; HBV, hepatitis B vaccine.

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From the 1930s to the late 1990s, many routinely administered vaccines in the United States contained thimerosal, an organic compound that is 49% mercury (Hg) by weight and is metabolized to ethylmercury and thiosalicylate. To meet Food and Drug Administration guidelines, thimerosal was added to vaccines to prevent bacterial and fungal contamination of multidose vaccine vials (except live viral vaccines).[1] Another organic Hg compound, methylmercury, has been found in studies of fish and grain ingestion to affect human neurologic and renal systems.[2–4] These studies, along with studies of prenatal Hg exposure, have been used by regulatory agencies to develop guidelines on exposure limits for methylmercury, the most stringent of which was set by the Environmental Protection Agency (EPA).[5–8] During a Food and Drug Administration review of Hg and other metals in drugs, it was determined that some infant immunization schedules that use thimerosal-containing vaccines (TCVs) adopted in 1991 may have exceeded the 1995 EPA guidelines for exposure to organic Hg (1 μg/kg/d vs 3 μg/kg/d in the previous 1985 EPA guidelines).[1,9–11] In July 1999, the American Academy of Pediatrics and the US Public Health Service recommended removing thimerosal from childhood vaccines as soon as possible as a precautionary measure.[12–14]

Although oral ingestion of organic Hg has been studied, information concerning the effects of parenteral exposure to these compounds in humans is limited to a few case reports,[15–18] none of which involved exposure from vaccines. Vaccines, however, constitute a nearly universal exposure for children in the United States and most other countries. To evaluate the theoretical concerns of the potentially toxic effects of thimerosal in vaccines, we studied neurodevelopmental outcomes among a large group of children with documented exposure to varying levels of thimerosal from vaccinations in several health maintenance organizations (HMOs).

## METHODS

The study was conducted in 2 phases. In phase I, a number of neurodevelopmental disorders were identified a priori as possibly related to ethylmercury exposure.[2–5,7] In this phase, using primar-

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ily preexisting HMO administrative databases collected for the Vaccine Safety Datalink (VSD) project, we screened for potential associations between these disorders and cumulative thimerosal exposure by 1, 3, and 7 months of age.[10] In the second phase, we attempted to confirm selected positive associations seen in phase I between thimerosal exposure and these outcomes in another independent cohort of HMO children (phase II) with similar largely preexisting HMO administrative data. Because of the smaller size of this second cohort, we were able to evaluate only the most common outcomes associated with thimerosal in phase I.

### Study Participants

For phase I, we studied a cohort of infants from the VSD project, which was created in 1991 by the National Immunization Program of the Centers for Disease Control and Prevention (CDC). The VSD methods have been described previously.[19–21] The project links medical event information, specific vaccine history (including manufacturer and lot number), and selected demographic information from the computerized databases of several HMOs. Because most of the neurodevelopmental outcomes of interest would have been cared for only in the outpatient setting, we restricted our analyses to children who were born from January 1992 through December 1998 at the 2 HMOs (HMO A and HMO B) with the most complete computerized outpatient data. At HMO A, clinic data for outcomes were available throughout the study period; for HMO B, clinic data were available starting in January 1995. For both HMOs, children had follow-up data through the end of 2000. For phase II, we used computer databases similar to those of the VSD to study children in a third HMO (HMO C), where data were available on children who were born from January 1991 through December 1997, with follow-up through May 1998.

To capture all vaccinations in the first year of life, we restricted the cohorts to children who were born into the HMO and remained enrolled continuously for the first year of life. To be certain that we studied children who actually received most of their primary care through the HMO, we excluded children who did not have documentation in the HMO databases of at least 2 polio vaccines by the age of 1 year.

We excluded from the main analysis infants with low birth weight (LBW) of <2500 g and those with a diagnosis of a congenital or severe perinatal disorder or born to mothers with serious medical problems of pregnancy (Appendix 1). We performed a separate analysis of infants with birth weights between 1500 and 2499 g.

### Exposure Assessment

We assessed cumulative exposure at 1, 3, and 7 months of life, when the exposure burden relative to body weight was highest. During the years of the study, the HMOs routinely used multidose vials for the vaccines of interest, and the exposure estimates were based on the mean Hg content of each vaccine in multidose vials (Table 1).

### Outcome Assessment

We identified the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM) diagnosis codes in the VSD database for HMOs A and B and the Costar codes in HMO C's database that were associated with the a priori selected neurodevelopmental disorders. The automated administrative databases that were used for this study included diagnoses made in the clinic, emergency department, and hospital.[19,20] Because a diagnosis of most of these conditions in the first year of life may be less reliable than later diagnoses, we included only diagnoses that were made past the age of 12 months.

Speech and language disorders were important outcomes, but coding practices for these conditions varied by HMO. At HMO B, separate codes for language delay (ICD-9 315.31) and speech delay (315.39) were used. At HMO A, only the code for speech delay was used, and there were no language delay codes. At HMO C, the Costar code was for combined language and speech disorders. Theoretically, the "language delay" code should be indicative of problems with expressive language development (eg, vocabulary, tense, word recall, sentence length and complexity) and the "speech delay" code should indicate difficulties or delays in development of speech sounds appropriate for age (eg, substituting one sound for another, omission of final consonants). The distinction between the diagnostic terms attention-deficit/hyperactivity disorder and attention-deficit disorder (ADD) can also be confusing. In this report, we use the term ADD to be consistent with the ICD-9 code (314.0) that we used in our analyses.

To assess the validity of the computerized diagnoses, we reviewed medical charts for selected diagnoses codes ascertained through 1998. For speech and language delay, autism, and ADD, we reviewed the medical charts of all 618 children in HMOs A and B and 826 children in HMO C with at least 2 automated diagnoses of speech delay, and a sample of 377 children in HMOs A and B and 100 children in HMO C with at least 1 automated diagnosis of ADD, and 120 children in HMOs A and B with at least 1 automated diagnosis of autism. For verification, we required documentation in the medical record that the diagnosis was made by an appropriate clinical or behavioral specialist.

### Statistical Analyses

In the primary analyses, relative risks (RRs) were calculated for the cumulative exposure to thimerosal by 1, 3, and 7 months of age. Because of power considerations, we decided a priori to perform an evaluation only of the cumulative effect of thimerosal exposure on the risk of outcomes with at least 50 or more cases. We estimated RRs separately for each HMO, using proportional hazards models stratified by sex and year and month of birth at HMO A and by sex and year and month of birth and clinic most

**TABLE 1.** Hg Exposure From TCVs for Children Following the Recommended Immunization Schedule in the First 7 Months of Life, HMOs A and B, 1992–1999

| Age at Exposure | Vaccines (Dose) | Total Hg Dose in the Period | Cumulative Hg Dose at End of the Period |
|---|---|---|---|
| First mo | HBV (First dose) | 12.5 μg | 12.5 μg |
| 2–3 mo | DTP and Hib (first dose)<br>HBV (second dose)* | 25, 37.5, 50, or 62.5 μg† | 37.5, 50, 62.5, or 75 μg† |
| 4–5 mo | DTP and Hib (second dose)<br>HBV (second dose)* | 25, 37.5, 50, or 62.5 μg‡ | 75 or 125 μg‡ |
| 6–7 mo | DTP (third dose)<br>Hib (third dose)<br>HBV (third dose) | 25, 50, or 62.5 μg§ | 112.5 or 187.5 μg§ |

DTP indicates combined diphtheria, tetanus, and Pertussis vaccine; Hib, *Haemophilus influenzae* type B vaccine.

* HBV second dose can be administered between months 1 and 4.

† Depending on whether DTP and Hib were given separately (both contain 25 μg of Hg) or as a combination vaccine (containing 25 μg of Hg) and the timing of the second HBV dose.

‡ Depending on whether DTP and Hib were given separately or as a combination vaccine and assuming that all 3 doses are given as combined or separate.

§ Depending on whether DTP and Hib were given separately or as a combination vaccine and assuming that all 3 doses are given as combined or separate and whether the third HBV dose was given before 7 months.

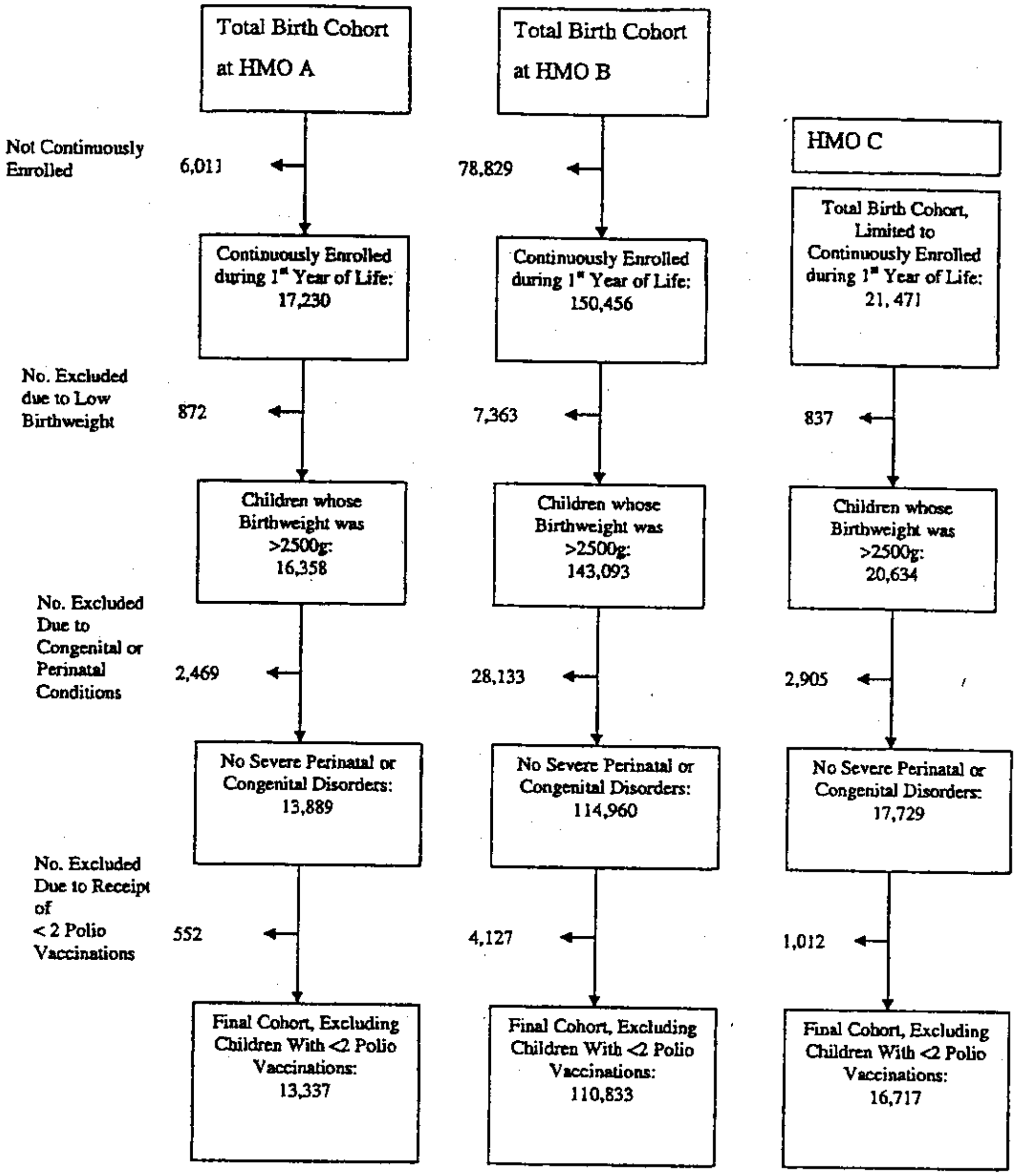


Fig 1. Creation of study cohorts at HMOs A, B, and C.

often visited at HMO B. The time variable in the models started at the first birthday for children in HMO A or at the first birthday or January 1, 1995 (whichever came later), for children at HMO B; for children in each HMO, the time of follow-up ended at the date of diagnosis or the last date of follow-up. Temporary disenrollment with reenrollment was allowed, but person-time and diagnoses were used only while the child was enrolled in the HMO. We used $P < .05$ to define statistical significance.

We were concerned that parents who had their children vaccinated on time (and therefore were more likely to have increased thimerosal exposure at each of the time periods studied) were also more likely to seek medical care for common pediatric ambulatory conditions. Support for this concern was provided by analyses indicating that in each year from 1994 to 1998, children who received >75 μg Hg in the first 7 months of life, compared with children who received 0 to 75 μg Hg in the first 7 months of life, had significantly more well child care visits and significantly more visits for "upper respiratory infections" in both the second and third years of life (Appendix 2). To try to control for health care-seeking behavior, we performed the analyses in phase I restricted to children who had made at least 1 visit to a clinic or an emergency department at the same month of age as cases. For phase II, this extent of health care visits data was not available in the analytic data set and no such adjustment for health care-seeking behavior was possible. We also were not able to make such adjustments in the subanalysis of LBW infants.

To simplify the presentation of the results given the large number of outcomes studied and the different exposure time periods that were assessed, we modeled exposure as a continuous variable with increments of 12.5 μg Hg. To illustrate the change in risk with each level of exposure and as a visual check of the linearity assumption made in analyses of exposure as a continuous variable, we also performed additional analyses in which we modeled exposure as a categorical variable. For these analyses, the exposure levels were 0 to 25 μg, 37.5 to 50 μg, and ≥62.5 μg at 3 months and 0 to 75 μg, 87 to 162.5 μg, and ≥175 μg at 7 months, respectively. We restricted these analyses to outcomes for which significant associations were found in the analysis of exposure as a linear variable and certain outcomes of particular interest (eg, ADD, autism).

In the analyses restricted to moderately LBW infants, we included children who weighed from 1500 to 2499 g at birth, were enrolled in the HMO in the first month of life and remained enrolled past 1 year of age, and had 2 or more polio vaccinations by 1 year. We were not able to maintain the other exclusion criteria listed in Appendix 1 because insufficient numbers would have remained in the analysis. The statistical analyses were stratified by

TABLE 2. Number of Children, Age at Diagnosis, and Proportion of Boys Diagnosed at HMOs A (1992–2000) and B (1995–2000)

| ICD-9 Code | Outcome | HMO A | | | HMO B | | |
|---|---|---|---|---|---|---|---|
| | | *N* | Age* | % Boys | *N* | Age* | % Boys |
| All children | | 13 337 | | 51 | 110 833 | | 50 |
| 299.0 | Autism | 21 | 49 | 90 | 202 | 44 | 80 |
| 299.8 | Other childhood psychosis | 20 | 54 | 70 | 108 | 55 | 94 |
| 307.0 | Stammering | 61 | 40 | 70 | 112 | 39 | 69 |
| 307.2 | Tics | 62 | 61 | 60 | 201 | 62 | 75 |
| 307.4 | Sleep disorders | 70 | 32 | 56 | 159 | 29 | 58 |
| 307.5 | Eating disorders | 20 | 33 | 30 | 82 | 26 | 54 |
| 313.8 | Emotional disturbances | 84 | 67 | 73 | 320 | 64 | 78 |
| 314.0 | ADD | 170 | 72 | 78 | 940 | 70 | 79 |
| 315.31 | Developmental language delay | 35 | 62 | 60 | 586 | 32 | 75 |
| 315.39 | Developmental speech delay | 694 | 38 | 68 | 1941 | 31 | 72 |
| 315.3 | Speech or language delay | 730 | 38 | 68 | 2288 | 31 | 72 |
| 315.4 | Coordination disorder | 83 | 35 | 64 | 26 | 56 | 73 |

* Median age at first diagnosis, in months.

HMO, year of birth, and sex and controlled for birth weight (250-g intervals).

## RESULTS

### Phase I: HMOs A and B

*Cohort Selection*

A total of 252 526 children (23 241 at HMO A and 229 285 at HMO B) were born into the 2 VSD HMOs (Fig 1). After all exclusion criteria were applied, the final study cohort size was 13 337 at HMO A and 110 833 at HMO B.

*Outcome Assessment*

At HMO A, 8 categories of neurodevelopmental disorders contained 50 or more children; in HMO B, there were 11 such categories (Table 2). The most frequent diagnoses were those of speech delay followed by ADD. As noted previously, there were substantive differences in the proportions of children at HMOs A and B who had a diagnosis of speech delay or of language delay. The median age at first diagnosis for the children within the study cohorts varied from 26 months for eating disorders to 72 months for ADD. For each category of neurodevelopmental disorders (with the exception of one), more boys than girls received a diagnosis of neurodevelopmental disorders. For the children whose charts were reviewed, the confirmation rates for speech delay, autism, and ADD were 81.6%, 92.3%, and 42.1% for HMO A and 66.8%, 81.3%, and 28.2% for HMO B, respectively.

*Risk Estimates*

Tables 3 and 4 show the adjusted RRs associated with cumulative thimerosal exposure by 1, 3, and 7 months of age. At HMO A, a significantly increased risk was seen only with cumulative exposure at 3 months and the diagnosis of tics. At HMO B, significantly increased risks were seen with cumulative exposure at 3 and 7 months and language delay.

In the categorical analyses of cumulative exposure at 3 months of age at HMO B (Table 5), there was a significant association between the highest level of exposure (≥62.5 micrograms) and language delay. For the categorical analyses of cumulative exposure at 7 months of age (Table 5), there was a borderline statistically significant negative association of speech delay with medium and high levels of thimerosal exposure at HMO A. There were no significant associations between exposure and ADD.

There were sufficient cases for analysis of autism only at HMO B. No significant associations were found with cumulative exposure at any age and risk for autism in either the continuous (Table 4) or the categorical analyses (Table 5).

TABLE 3. RRs by Increase of 12.5 μg of Hg Exposure From TCVs at HMO A

| Outcome | 1-Month Cumulative Hg | | 3-Month Cumulative Hg | | 7-Month Cumulative Hg | |
|---|---|---|---|---|---|---|
| | RR | 95% CI | RR | 95% CI | RR | 95% CI |
| Stammering | 0.89 | 0.40–1.97 | 1.18 | 0.74–1.89 | 1.17 | 0.97–1.41 |
| Tics | 1.25 | 0.47–3.29 | 1.89* | 1.05–3.38 | 1.12 | 0.93–1.34 |
| Sleep disorders | 0.79 | 0.38–1.61 | 0.93 | 0.71–1.21 | 1.08 | 0.95–1.24 |
| Emotional disturbances | 1.00 | 0.42–2.36 | 0.98 | 0.66–1.45 | 0.92 | 0.81–1.03 |
| ADD | 0.92 | 0.52–1.59 | 0.83 | 0.68–1.02 | 0.93 | 0.84–1.02 |
| Speech delay | 1.07 | 0.83–1.38 | 1.03 | 0.93–1.15 | 0.97 | 0.92–1.01 |
| Speech/language delay | 1.14 | 0.88–1.46 | 1.03 | 0.93–1.14 | 0.97 | 0.93–1.02 |
| Coordination disorders | 1.67 | 0.78–3.57 | 1.19 | 0.82–1.71 | 1.00 | 0.87–1.15 |

CI indicates confidence interval.
* $P < .05$.

TABLE 4. RRs by Increase of 12.5 μg of Hg Exposure From TCVs at HMO B

| Outcome | 1-Month Cumulative Hg | | 3-Month Cumulative Hg | | 7-Month Cumulative Hg | |
|---|---|---|---|---|---|---|
| | RR | 95% CI | RR | 95% CI | RR | 95% CI |
| Autism | 1.16 | 0.78–1.71 | 1.06 | 0.88–1.28 | 1.00 | 0.90–1.09 |
| Other child psychosis | 1.03 | 0.60–1.74 | 0.93 | 0.73–1.19 | 1.04 | 0.91–1.20 |
| Stammering | 0.61 | 0.33–1.14 | 1.10 | 0.86–1.41 | 1.06 | 0.93–1.21 |
| Tics | 0.85 | 0.55–1.30 | 0.95 | 0.78–1.15 | 1.09 | 0.98–1.21 |
| Sleep disorders | 1.24 | 0.80–1.93 | 1.15 | 0.95–1.39 | 1.09 | 0.99–1.19 |
| Eating disorders | 0.90 | 0.50–1.61 | 0.97 | 0.72–1.29 | 0.98 | 0.85–1.14 |
| Emotional disturbances | 0.76 | 0.54–1.07 | 1.02 | 0.88–1.18 | 1.01 | 0.93–1.10 |
| ADD | 0.90 | 0.74–1.10 | 1.01 | 0.93–1.11 | 1.02 | 0.97–1.07 |
| Language delay | 1.06 | 0.83–1.35 | 1.13* | 1.01–1.27 | 1.07* | 1.01–1.13 |
| Speech delay | 1.02 | 0.90–1.17 | 1.04 | 0.98–1.10 | 1.02 | 0.99–1.05 |
| Language/speech delay | 1.03 | 0.91–1.17 | 1.05 | 0.99–1.11 | 1.02 | 0.99–1.05 |

* $P < .05$.

TABLE 5. RRs by Category of Cumulative Hg Exposure at 3 and 7 Months

| Outcome | Hg (μg) | HMO A | | | | HMO B | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RR 95% CI | N | $\chi^2$ | P Value | RR 95% CI | N | $\chi^2$ | P Value |
| 3 Months | | | | | | | | | |
| | 0–25 | 1.00 | 61 | 0.13 | .94 | 1.00 | 106 | 2.28 | .32 |
| Speech delay | 37.5–50 | 1.04 (0.61–1.75) | 107 | | | 1.14 (0.91–1.44) | 1297 | | |
| | ≥62.5 | 1.09 (0.65–1.81) | 526 | | | 1.21 (0.93–1.58) | 538 | | |
| | 0–25 | | 1* | | | 1.00 | 24 | 5.44 | .07 |
| Language delay | 37.5–50 | | 6 | | | 1.44 (0.90–2.28) | 419 | | |
| | ≥62.5 | | 28 | | | 1.87 (1.08–3.23) | 143 | | |
| ADD | 0–25 | 1.00 | 5 | 4.36 | .11 | 1.00 | 55 | 0.33 | .85 |
| | 37.5–50 | 0.88 (0.27–2.79) | 60 | | | 1.00 (0.71–1.39) | 627 | | |
| | ≥62.5 | 0.41 (0.13–1.20) | 105 | | | 1.08 (0.72–1.61) | 258 | | |
| | 0–25 | | 1 | | | 1.00 | 11 | 1.84 | .40 |
| Autism | 37.5–50 | | 5 | | | 1.61 (0.77–3.34) | 158 | | |
| | ≥62.5 | | 15 | | | 1.38 (0.55–3.48) | 33 | | |
| 7 Months | | | | | | | | | |
| | 0–75 | 1.00 | 68 | 5.37 | .07 | 1.00 | 250 | 2.23 | .33 |
| Speech Delay | 87–162.5 | 0.58 (0.37–0.93) | 202 | | | 1.11 (0.95–1.30) | 1362 | | |
| | ≥175 | 0.58 (0.36–0.92) | 424 | | | 1.04 (0.82–1.32) | 329 | | |
| Language delay | 0–75 | | 1* | | | 1.00 | 75 | 2.34 | .31 |
| | 87–162.5 | | 10 | | | 1.20 (0.91–1.59) | 422 | | |
| | ≥175 | | 24 | | | 1.37 (0.87–2.14) | 89 | | |
| ADD | 0–75 | 1.00 | 2 | 2.33 | .31 | 1.00 | 101 | 2.51 | .28 |
| | 87–162.5 | 1.64 (0.32–8.28) | 82 | | | 1.22 (0.95–1.57) | 684 | | |
| | ≥175 | 1.19 (0.23–6.05) | 86 | | | 1.21 (0.83–1.76) | 155 | | |
| | 0–75 | | 1 | | | 1.00 | 37 | 1.08 | .58 |
| Autism | 87–162.5 | | 8 | | | 0.95 (0.62–1.46) | 148 | | |
| | ≥175 | | 12 | | | 0.65 (0.27–1.52) | 17 | | |

* No comparisons were made when fewer than 50 children received a diagnosis of a condition.

### Phase II: HMO C

#### *Cohort Selection*

A total of 21 471 children were born into HMO C and were also continuously enrolled for the first year of life (the numbers of the entire birth cohort, including those not continuously enrolled for the first year of life, were unavailable). After children who did not receive at least 2 polio vaccinations in the first year or who had LBW or a serious congenital or perinatal anomaly were excluded, the final study cohort size was 16 717 (Fig 1).

#### *Outcome Assessment*

A total of 1134 children had a speech/language delay, 91 children had stammering, 499 children had sleep disorders, and 97 children had ADD from the computerized clinic records. The median age at first diagnosis was 24 months for speech or language disorder, 50 months for ADD, 35 months for stammering, and 19 months for sleep disorder, and similar to phase 1, there was a male excess for each disorder. Among the children for whom medical records were reviewed, we confirmed 647 (78%) and 44 (44%) of the automated diagnoses of speech or language delay and ADD, respectively.

#### *Risk Estimates*

There were no significant associations between cumulative thimerosal exposures at 1, 3, or 7 months of age and speech/language disorder, ADD, stammering, or sleep disorder (Table 6).

### Analysis of LBW Infants: HMOs A, B, and C

In the subanalysis of LBW infants, a limited number of outcomes could be evaluated because of sample size constraints. Restricting the analyses to conditions with at least 50 cases, we evaluated risk for cumulative mercury exposure at 3 and 7 months by

TABLE 6. RRs by Increase of 12.5 $\mu$g of Hg Exposure From TCVs at HMO C

| Outcome | 1-Month Cumulative Hg | | 3-Months Cumulative Hg | | 7-Months Cumulative Hg | |
|---|---|---|---|---|---|---|
| | RR | 95% CI | RR | 95% CI | RR | 95% CI |
| Stammering | 0.77 | 0.47–1.26 | 0.97 | 0.78–1.20 | 0.99 | 0.88–1.10 |
| Tics | 0.93 | 0.45–1.92 | 1.26 | 0.81–1.94 | 1.18 | 0.97–1.42 |
| Sleep disorders | 0.97 | 0.79–1.19 | 1.02 | 0.92–1.13 | 1.05 | 0.99–1.11 |
| ADD | 0.88 | 0.53–1.48 | 0.96 | 0.79–1.18 | 0.96 | 0.87–1.05 |
| Speech/language delay | 0.91 | 0.79–1.04 | 0.96 | 0.90–1.02 | 0.98 | 0.94–1.01 |

12.5-$\mu$g increase (Table 7). We were able to evaluate the combined outcome of speech or language delay at all 3 HMOs and ADD at HMO B. We found no statistically significant increased risks for either outcome.

## DISCUSSION

In this analysis using computerized HMO databases to screen for possible associations between exposure to thimerosal in infant vaccines and neurodevelopmental outcomes, we did not find evidence of a clear association between thimerosal and specific neurodevelopmental disorders. In the first phase of our study, we observed an association between thimerosal exposure and some of the neurodevelopmental disorders screened, most notably between cumulative thimerosal exposure by 3 and 7 months of age and speech and language disorders at 1 HMO, and also an association between cumulative thimerosal exposure by 3 months of age in 1 HMO and tic disorder. The results between HMOs, however, were inconsistent. Our study encompassed a large number of separate analyses and, by chance alone, at least some associations would be expected to be statistically significant. We did not adjust the level of statistical significance of our estimates for the multiple comparisons made but chose instead to attempt to confirm our positive findings in an independent third HMO. In the second phase of this study, no associations that had been seen previously in either of the first 2 HMOs were detected at the third HMO.

The discrepant findings have several possible explanations, including differences in outcome ascertainment. HMO B is the only HMO in our study where speech therapy is not covered by the health plan. Because such therapy is not provided, primary care providers in this HMO may have screened less aggressively for speech or language disorders among young children. Thus, parental concern may have been a more important factor in the ascertainment of these disorders. If parents at this HMO who were more concerned about subtle neurodevelopmental delays were also more likely to adhere to a timely vaccination schedule, then ascertainment bias might have resulted in falsely elevated estimates of the association between thimerosal and these disorders. We attempted to control for differences in health care-seeking behavior by matching on clinic visits. Nevertheless, some significant associations remained for language delay.

The biological plausibility of the small doses of ethylmercury present in vaccines leading to increased risks of neurodevelopmental disorders is uncertain. The effect of organic Hg on neurologic development has been the focus of several studies.[5,7,22–24] Two prospective cohort studies of prenatal exposure to methylmercury from fish consumption have resulted in conflicting findings. In the Seychelles, Davidson et al[5,25] found no effect of pre- or postnatal methylmercury exposure on the neurologic development of 711 children at 66 months of age. In the Faroe Islands, Grandjean and colleagues[7,9,26] found an adverse effect of prenatal exposure to methylmercury on attention, language, and memory at 7 years of age among 917 children. Attention was also found to be inversely related to hair Hg concentrations in Amazonian children aged 7 to 12 years,[23] and speech retardation by 24 months was related to maternal hair Hg concentrations in Iraqi children.[27]

All of these and other studies involved ingested methylmercury, and their relevance to our study of ethylmercury bolus exposure by injection of TCVs is unknown. The magnitudes of Hg exposure in these other studies were also much higher than Hg exposure from vaccines. For example, blood Hg levels

TABLE 7. RRs by Increase of 12.5 $\mu$g of Hg Exposure from TCVs for Selected Outcomes Among Moderately Low Birth Weight Infants (1500–2499 g)

| Outcome | HMO | Cases ($n$) | 3-Months Cumulative Hg | | 7-Months Cumulative Hg | |
|---|---|---|---|---|---|---|
| | | | RR | 95% CI* | RR | 95% CI |
| Speech or language delay | A | 55 | 1.09 | 0.86–1.37 | 1.04 | 0.92–1.17 |
| | B | 194 | 0.93 | 0.82–1.06 | 0.98 | 0.91–1.05 |
| | C | 65 | 0.97 | 0.79–1.19 | 1.04 | 0.90–1.19 |
| ADD | B | 64 | 0.99 | 0.75–1.29 | 0.99 | 0.83–1.17 |

* RR (95% CI) from proportional hazards regression models stratified by year of birth and sex, and adjusted for birth weight (250-g increments). The ADD results were also stratified by usual clinic and controlled for health care–seeking behavior.

after hepatitis B vaccine (HBV) in newborns, as measured by Stajich and colleagues,[7,9,22,28,29] although significantly elevated, were far below "no effect" levels as determined by the studies in the Faroe and Seychelles Islands. The results of a recently published study suggest that ethylmercury from thimerosal is metabolized and cleared from children more rapidly than methylmercury.[30] The Immunization Safety Review Committee of the Institute of Medicine concluded that although the evidence is indirect and incomplete, the hypothesis that TCVs could be associated with neurodevelopmental disorders is biologically plausible.[31]

Our use of automated databases has a number of limitations. As most vaccines used in the study population were either thimerosal-free throughout our study period (eg, polio) or thimerosal containing throughout our study period (eg, multidose HBV), our main analyses did not differentiate between the effect of thimerosal and other vaccine components. For example, we did not differentiate potential effects of thimerosal from those of whole-cell pertussis vaccine, which has been associated with an increased risk of encephalopathy. Encephalopathy after pertussis vaccination, however, is rare and unlikely to have had a meaningful impact on our results.[32]

To try to isolate the effects of thimerosal from other vaccine constituents, we performed a subanalysis comparing risks associated with diphtheria-tetanus-whole cell pertussis vaccine or diphtheria-tetanus-acellular pertussis vaccine and *Haemophilus influenzae* type b vaccine given separately or combined (Appendix 3). The 2 vaccination regimens included the same vaccine antigens but differed by Hg content (25 μg for the combined vaccine vs a total of 50 μg when the 2 vaccines were given separately). Only at HMO B were both the combined and separate products used. In the analyses of speech and language delay and ADD with cumulative exposure by 3 months, we did not find any statistically significant increased risks associated with increase in Hg exposure when the 2 vaccines were given separately compared with combined.

We evaluated the effect of the study exclusion criteria to determine whether they had an undue influence on our study findings. For the outcomes of speech delay and/or language delay, there was no appreciable effect on the observed RR of any of the exclusion criteria (Appendix 4). A similar analysis for autism also found no appreciable effect of the exclusion criteria (data not shown).

Our data may have been subject to misclassification errors in both exposure assessment and case ascertainment. Some vaccinations, particularly the neonatal HBV dose, may not have been captured completely. Mullooly et al[33] evaluated reliability of automated vaccination data in the VSD and estimated that 18% and 2% of HBV may have been missed at HMOs A and B, respectively. For other TCVs, the proportions missed were estimated to be 2% for both diphtheria-tetanus-whole cell pertussis and *Haemophilus influenzae* type b at HMO B and 10% and 9% for the same respective vaccines at HMO A. No specific evaluations of the accuracy of the automated records have been conducted at HMO C, but the accuracy is believed to be high, as the computerized records represent the sole medical record.

For case ascertainment, we used ICD-9 codes at 2 HMOs and Costar codes at the third. The low confirmation rates for ADD illustrate the potentially low positive predictive value of these codes, which could have limited our ability to find an association with this outcome. For other disorders, such as autism, the confirmation rate of the computerized codes was reasonably good. In a subanalysis (not shown), we found consistent results based on computerized codes compared with analyses based on a smaller sample of subjects with autism, ADD, and speech and language disorders whose medical records were reviewed and diagnoses confirmed, suggesting that the reliance on automated data did not introduce appreciable bias.

We were not able to control completely for potentially confounding factors. Clinic identity was unavailable from HMOs A and C and therefore could not be controlled for in the analysis. The variable that denoted which clinic a child attended acted as an appreciable confounder in the analyses at HMO B, and its absence from the other analyses represents a legitimate concern. In terms of the ability for this study to address the effect of other, potentially confounding environmental influences, the HMO databases did not contain information on potential predisposing factors for neurodevelopmental disorders, such as maternal smoking, lead exposure, or fish consumption. However, it is not obvious how these factors would be related to the child's vaccination status and thus confound the results.

LBW is a particularly important potentially confounding factor because LBW infants (especially those severely premature) are less likely to be vaccinated on time,[34] and they are also at increased risk for neurodevelopmental disorders.[35] We dealt with this potential bias by excluding LBW infants from the main analysis. Because LBW infants may be especially susceptible to thimerosal exposure as a result of their higher exposure doses relative to weight and their less developed nervous systems, we performed a subanalysis restricted to infants with moderately low birth weights (1500–2499 g). We were able to evaluate ADD and speech or language disorders and did not find significant increased risks associated with increasing thimerosal exposure.

## CONCLUSIONS

In our analyses of computerized HMO data, we found no consistent significant associations between TCVs and neurodevelopmental outcomes. In the first phase of our study, we found an association between exposure to Hg from TCVs and some of the neurodevelopmental outcomes screened. In the second phase, these associations were not replicated for the most common disorders in an independent population. Although the lack of consistency between the 2 phases argues against a thimerosal effect, we believe that additional investigation is required because of the widespread exposure from vaccinating virtually the entire birth cohort of the United States and the importance of speech and language disorders among children and adolescents. For elucidating further whether a causal association exists between thimerosal exposure and neurodevelopmental conditions, additional studies with different designs will be needed. A study with neuropsychological testing of children with different thimerosal exposures would address one of the main limitations of our present study: the reliance on administrative medical records for outcome assessment. Although such a study might also avoid ascertainment bias that may have affected the results of this study, it might still be susceptible to confounding if factors that influence parents' decision to have their children vaccinated timely are also related to their children's neurodevelopment. Although this bias could conceivably be eliminated by conducting a randomized controlled trial, such a trial would not be ethically feasible given current recommendations that thimerosal not be included in routine infant vaccines. The best alternative is to evaluate the development of children who were enrolled in previous randomized vaccine trials in which the vaccines contained similar antigens but differed by thimerosal content.

APPENDIX 1. Perinatal Exclusion Codes Used in the Thimerosal Screening Analyses

| | |
|---|---|
| 740.* | Anencephalus, craniorachischisis, iniencephaly |
| 741.* | Spina bifida |
| 742.* | Encephalocele, microcephlus, other brain and spinal cord anomalies |
| 745.* | Cardiac defects including ventricular septal defect |
| 746.* | Other congenital heart defects |
| 747.* | Anomalies of aorta, other arteries, veins |
| 748.* | Various abnormalities of nose, lung, respiratory abnormalities |
| 749.* | Cleft palate and cleft lip |
| 750.* | Tongue and mouth abnormalities |
| 751.* | Abnormalities of intestine, pancreas, other digestive |
| 753.0 | Renal agenesis |
| 756.6 | Anomalies of diaphragm |
| 756.7 | Abdominal wall abnormalities |
| 758.* | Chromosomal abnormalities |
| 759.7 | Multiple congenital anomalies not elsewhere coded |
| 759.9 | Congenital anomaly not otherwise specified |
| 760.* | Maternal condition affecting fetus including maternal injury, hypertension, drugs |
| 761.* | Maternal complication affecting newborn including premature rupture of membrane |
| 764.* | Slow fetal growth, malnutrition, light for gestational age |
| 765.* | Disorders related to short gestation and unspecified low birth weight |
| 767.* | Birth trauma including scalp injury |
| 768.* | Intrauterine asphyxia, fetal distress during labor |
| 769.* | Respiratory distress syndrome |
| 770.* | Newborn respiratory condition |
| 772.1 | Intraventricular hemorrhage |
| 772.2 | Subarachnoid hemorrhage |
| 773.* | Newborn hemolytic disease |
| 775.* | Newborn endocrinological disease |
| 776.2 | Disseminated intravascular coagulation |
| 779.* | Other perinatal condition including convulsion (.0), feeding problems (.3) |

Note: 760.* was not used as an exclusion at HMO C.

APPENDIX 2. HMO B: Mean Number of Outpatient Visits for URI (ICD 460–466) and Well-Child Visits (V20*, V70.0, V70.3, V70.5, V70.9) in Second Year of Life by Year of Birth and Estimated Amount of Hg Received In First 7 Months of Life (0–75 vs >75 μg)

| Year | μg | *N* | URI | | | | Well-Child Visit | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | *t* Test | *P* Value | Mean | SD | *t* Test | *P* Value |
| 1994 | 0–75 | 2757 | 1.76 | 1.93 | −4.78 | <.0001 | 1.99 | 1.26 | −15.6 | <.0001 |
| | ≥75 | 11 072 | 1.96 | 2.00 | | | 2.42 | 1.36 | | |
| 1995 | 0–75 | 2660 | 1.59 | 1.77 | −3.56 | .0004 | 1.90 | 1.18 | −14.2 | <.0001 |
| | ≥75 | 11 405 | 1.73 | 1.84 | | | 2.26 | 1.20 | | |
| 1996 | 0–75 | 2563 | 1.60 | 1.76 | −5.48 | <.0001 | 1.79 | 1.08 | −15.6 | <.0001 |
| | ≥75 | 12 059 | 1.82 | 1.94 | | | 2.16 | 1.14 | | |
| 1997 | 0–75 | 1222 | 1.45 | 1.71 | −5.72 | <.0001 | 1.76 | 1.03 | −11.6 | <.0001 |
| | ≥75 | 13 486 | 1.75 | 1.82 | | | 2.12 | 1.11 | | |
| 1998 | 0–75 | 947 | 1.60 | 1.81 | 0.06 | .9540 | 1.83 | 1.06 | −7.9 | <.0001 |
| | ≥75 | 13 643 | 1.60 | 1.75 | | | 2.12 | 1.13 | | |

SD indicates standard deviation.
* Children followed continuously >2 years since birth.
Note: Restricting to children with ≤9 visits did not change the results.

APPENDIX 3. RR Associated With Cumulative Hg Exposure by 3 Months of Age for Selected Outcomes for Children Receiving Separate DTP/DTaP and Hib Vaccines Compared with Children Receiving Combined Vaccines, HMO B

| Outcome | *N* | No. of Cases | RR | 95% CI |
|---|---|---|---|---|
| Speech delay | 29 990 | 547 | 1.04 | 0.85–1.27 |
| Language delay | 29 845 | 167 | 1.29 | 0.91–1.82 |
| ADD | 13 985 | 315 | 0.70* | 0.52–0.95 |

* $P < .05$.

Note: HMO B started using a combined DTP and Hib vaccine in early 1993 and then replaced it with separate DTaP and Hib vaccinations in 1997. The analysis compared the risk among children who received a DTP (or DTaP) and Hib before 3 months of age in a single combination vaccine with those who received the vaccines separately. The analyses were stratified on sex, year of birth, and clinic but not on month of birth (as a result of the rapid change-over of use of vaccine, there was a lack of overlap by month of birth). The RR is expressed as the higher thimerosal group (separate vaccines) compared with the lower group (combined vaccine).

APPENDIX 4. Effects of Applying Exclusion Criteria

| | HMO A 7 Months Speech/ Language Delay | | | HMO B 7 Months Speech Delay | | |
|---|---|---|---|---|---|---|
| | Cases | RR | 95% CI | Cases | RR | 95% CI |
| All | 1115 | 0.99 | 0.96–1.03 | 3477 | 1.00 | 0.98–1.02 |
| I | 1056 | 0.99 | 0.96–1.03 | 2866 | 1.01 | 0.98–1.03 |
| II | 969 | 1.00 | 0.96–1.03 | 2654 | 1.01 | 0.99–1.04 |
| III | 742 | 0.99 | 0.95–1.03 | 1971 | 1.01 | 0.99–1.04 |
| IV | 730 | 0.97 | 0.93–1.02 | 1941 | 1.02 | 0.99–1.05 |

I Children not continuously enrolled during the first year of life excluded.
II LBW children excluded.
III Children with any condition in Appendix 1 excluded.
IV Children who received <2 polio vaccinations in the first year of life excluded.

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## REFERENCES

1. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics*. 2001;107:1147–1154
2. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Mercury* (Update). Atlanta, GA: Agency for Toxic Substances and Disease Registry; 1999
3. Bakir F, Damluji SF, Amin-Zaki L, et al. Methylmercury poisoning in Iraq. *Science*. 1973;181:230–241
4. Igata A. Epidemiological and clinical features of Minamata disease. *Environ Res*. 1993;63:157–169
5. Davidson PW, Myers GJ, Cox C, et al. Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. *Neurotoxicology*. 1995;16:677–688
6. Mahaffey KR, Rice G. *An assessment of Exposure to Mercury in the United States: Mercury Report to Congress*. Washington, DC: US Environmental Protection Agency; 1997
7. Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol*. 1997;19:417–428
8. WHO. *Environmental Health Criteria: Mercury*. Geneva, Switzerland: World Health Organization; 1976.
9. US Environmental Protection Agency. *Mercury Study Report to Congress. Volume V: Health Effects of Mercury and Mercury Compounds*. Washington, DC: Office of Air Quality Planning and Standards and Office of Research and Development; 1997
10. 21 USC, 397 Section 413; 2001
11. Verstraeten T, Gu D, Chen R. Exposure to Ethylmercury From Thimerosal Containing Vaccines in Infants. Presented at the 127th Annual Meeting of the American Public Health Association; November 9, 1999; Chicago, IL
12. Thimerosal in vaccines: a joint statement of the American Academy of Pediatrics and the Public Health Service. *Neonatal Netw*. 1999;18:65–72
13. Centers for Disease Control and Prevention. Recommendations regarding the use of vaccines that contain thimerosal as a preservative. *JAMA*. 1999;282:2114–2115
14. Centers for Disease Control and Prevention. Availability of hepatitis B

vaccine that does not contain thimerosal as a preservative. *JAMA*. 1999;282:1219–1220
15. Jessen P, Verdiere B, Moriet G, Boule D, Rhaoui A, Fraisse F. [An original mechanism of acute mercury poisoning: ritual injection]. *Presse Med*. 1991;20:1625–1626
16. Lowell JA, Burgess S, Shenoy S, Curci JA, Peters M, Howard TK. Mercury poisoning associated with hepatitis-B immunoglobulin. *Lancet*. 1996;347:480
17. Netscher DT, Friedland JA, Guzewicz RM. Mercury poisoning from intravenous injection: treatment by granuloma resection. *Ann Plast Surg*. 1991;26:592–596
18. Zosin C, Manescu N, Gluhovschi G, Nicolcioiu M, Golea O, Bignion H. [Acute mercury poisoning due to intravenous injection of metallic mercury]. *MMW Munch Med Wochenschr*. 1977;119:1537–1538
19. Chen RT, Glasser JW, Rhodes PR, et al. Vaccine Safety Datalink project: a new tool for improving vaccine safety monitoring in the United States. The Vaccine Safety Datalink Team. *Pediatrics*. 1997;99:765–773
20. Chen RT, DeStefano F, Davis RL, et al. The Vaccine Safety Datalink: immunization research in health maintenance organizations in the USA. *Bull World Health Organ*. 2000;78:186–194
21. Wassilak SG, Glasser JW, Chen RT, Hadler SC. Utility of large-linked databases in vaccine safety, particularly in distinguishing independent and synergistic effects. The Vaccine Safety Datalink Investigators. *Ann N Y Acad Sci*. 1995;754:377–382
22. Crump KS, Van Landingham C, Shamlaye C, et al. Benchmark concentrations for methylmercury obtained from the Seychelles Child Development Study. *Environ Health Perspect*. 2000;108:257–263
23. Grandjean P, White RF, Nielsen A, Cleary D, De Oliveira Santos EC. Methylmercury neurotoxicity in Amazonian children downstream from gold mining. *Environ Health Perspect*. 1999;107:587–591
24. Steuerwald U, Weihe P, Jorgensen PJ, et al. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *J Pediatr*. 2000;136:599–605
25. Davidson PW, Myers GJ, Cox C, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA*. 1998;280:701–707
26. Grandjean P, Budtz-Jorgensen E, White RF, et al. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am J Epidemiol*. 1999;150:301–305
27. Marsh DO, Myers GJ, Clarkson TW, Amin-Zaki L, Tikriti S, Majeed MA. Fetal methylmercury poisoning: clinical and toxicological data on 29 cases. *Ann Neurol*. 1980;7:348–353
28. Grandjean P, Weihe P, White RF, Debes F. Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. *Environ Res*. 1998;77:165–172
29. Stajich GV, Lopez GP, Harry SW, Sexson WR. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. *J Pediatr*. 2000;136:679–681
30. Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thimerosal: a descriptive study. *Lancet*. 2002;360:1737–1741
31. Stratton K, Gable A, McCormick MC, eds. *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders. Institute of Medicine. Immunization Safety Review Committee*. Washington, DC: National Academy Press; 2001
32. Howson CP, Howe CJ, Fineberg HV, eds. *Adverse Effects of Pertussis and Rubella Vaccines. Institute of Medicine*. Washington, DC: National Academy Press; 1991
33. Mullooly J, Drew L, DeStefano F, et al. Quality of HMO vaccination databases used to monitor childhood vaccine safety. *Am J Epidemiol*. 1999;149:186–194
34. Davis RL, Rubanowice D, Shinefield HR, et al. Immunization levels among premature and low-birth-weight infants and risk factors for delayed up-to-date immunization status. *JAMA*. 1999;282:547–53
35. Anderson P, Doyle LW. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *JAMA*. 2003;289:3264–3272

## MARGARET MEAD'S EARLY EDUCATION

"Kindergarten passed muster among the pedagogically enlightened women of the family. Margaret attended for two years. But from then until fourth grade, she had no official schooling. When she finally did show up in class it was only for half days, and she went armed with instructions from her parents that she was to be permitted to leave whenever she liked. When nine-year old Margaret decided to begin a diary, her first entry showed her to be very much the product of her eclectic yet ever so self-conscious rearing: 'I'm not sure that I won't miss days some times, for I am not very regalar.' At adolescence, she was swept up in a joint project with her mother . . . the two of them went off to study Italian immigrant children, aiming to find out how language affected IQ scores."

Hulbert A. *Raising America*. New York: Knopf; 2003

Submitted by Student

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World Health Organization

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# Statement on thiomersal

**The Global Advisory Committee on Vaccine Safety concludes that there is no evidence of toxicity in infants, children or adults exposed to thiomersal (containing ethyl mercury) in vaccines.**

In 1999, concerns were raised in the United States about exposure to mercury following immunization. This was based on the realization that the cumulative amount of mercury in the infant immunization schedule potentially exceeded the recommended threshold set by the United States government for methyl mercury. However thiomersal, the preservative in some vaccines, contains ethyl mercury not methyl mercury. The Global Advisory Committee on Vaccine Safety (GACVS) first assessed this issue at a special meeting in August 2000. The Committee review has been ongoing since then.

Expert consultation and data presented to the GACVS on 20-21 June 2002 indicate that the pharmacokinetic profile of ethyl mercury is substantially different from that of methyl mercury. The half-life of ethyl mercury is short (less than one week) compared to methyl mercury (1.5 months) making exposure to ethyl mercury in blood comparatively brief. Further, ethyl mercury is actively excreted via the gut unlike methyl mercury that accumulates in the body. Two independently-conducted epidemiological studies have been completed in the United Kingdom. These studies further support the safety of thiomersal-containing vaccines in infants in the amounts used in existing vaccines.

During a recent consultation held on 11-12 June 2003, GACVS reviewed available information on an ongoing thiomersal pharmacokinetic study in macaque monkeys; and a study on the suitability of thiomersal-free vaccines for multidose policy (assessed by retained sterility for up to 30 days).

On the basis of the foregoing, the GACVS concluded that the latest pharmacokinetic and developmental studies do not support concerns over safety of thiomersal (ethyl mercury) in vaccines. The Committee has concluded, and advises accordingly, that there is no reason on grounds of safety to change current immunization practices with thiomersal-containing vaccines, since the benefit outweighs any unproven risks. However, data for well-nourished neonates born at term cannot necessarily be extrapolated to preterm or malnourished infants. The latter studies would be difficult to conduct. GACVS encourages further research to be done bearing in mind the special difficulties in conducting studies in at-risk subjects.

The GACVS will continue to review the evidence and any epidemiological data that might emerge from on-going studies.

The GACVS is a scientific advisory body established by WHO to provide a reliable and independent scientific assessment of vaccine safety issues in order to respond promptly, efficiently and with scientific rigour to such issues. Membership includes experts from around the world in the fields of epidemiology, paediatrics, internal medicine, pharmacology and toxicology, infectious diseases, public health, immunology and autoimmunity, drug regulation, and safety.